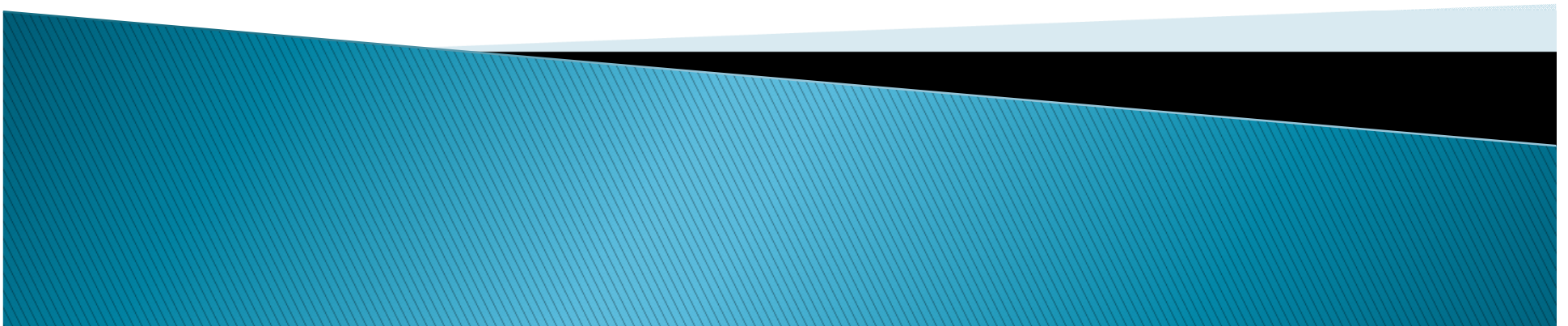


Genetic Disorders

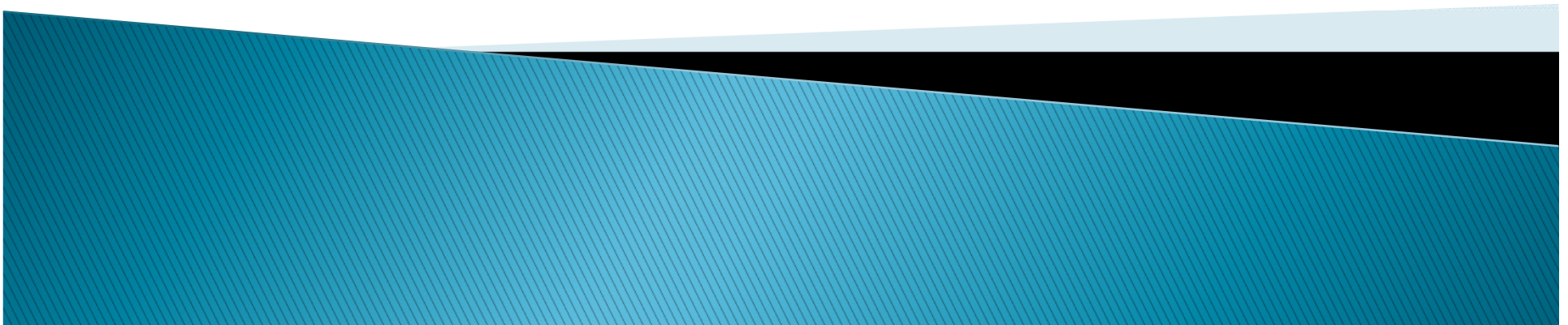
Dr Shilpa Goyal



Genetic disorders

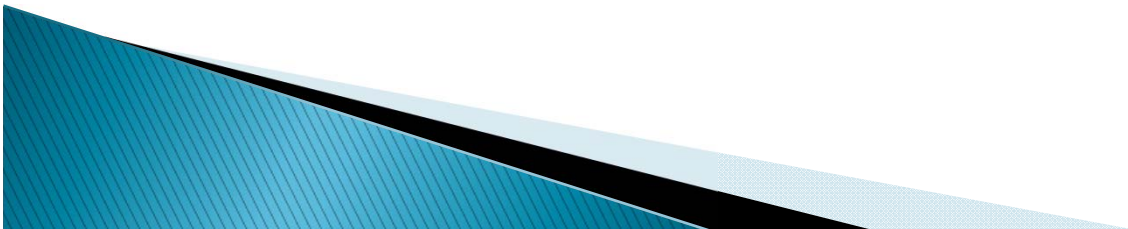
Overview, mendelian disorders and pattern
of inheritance

Class 1



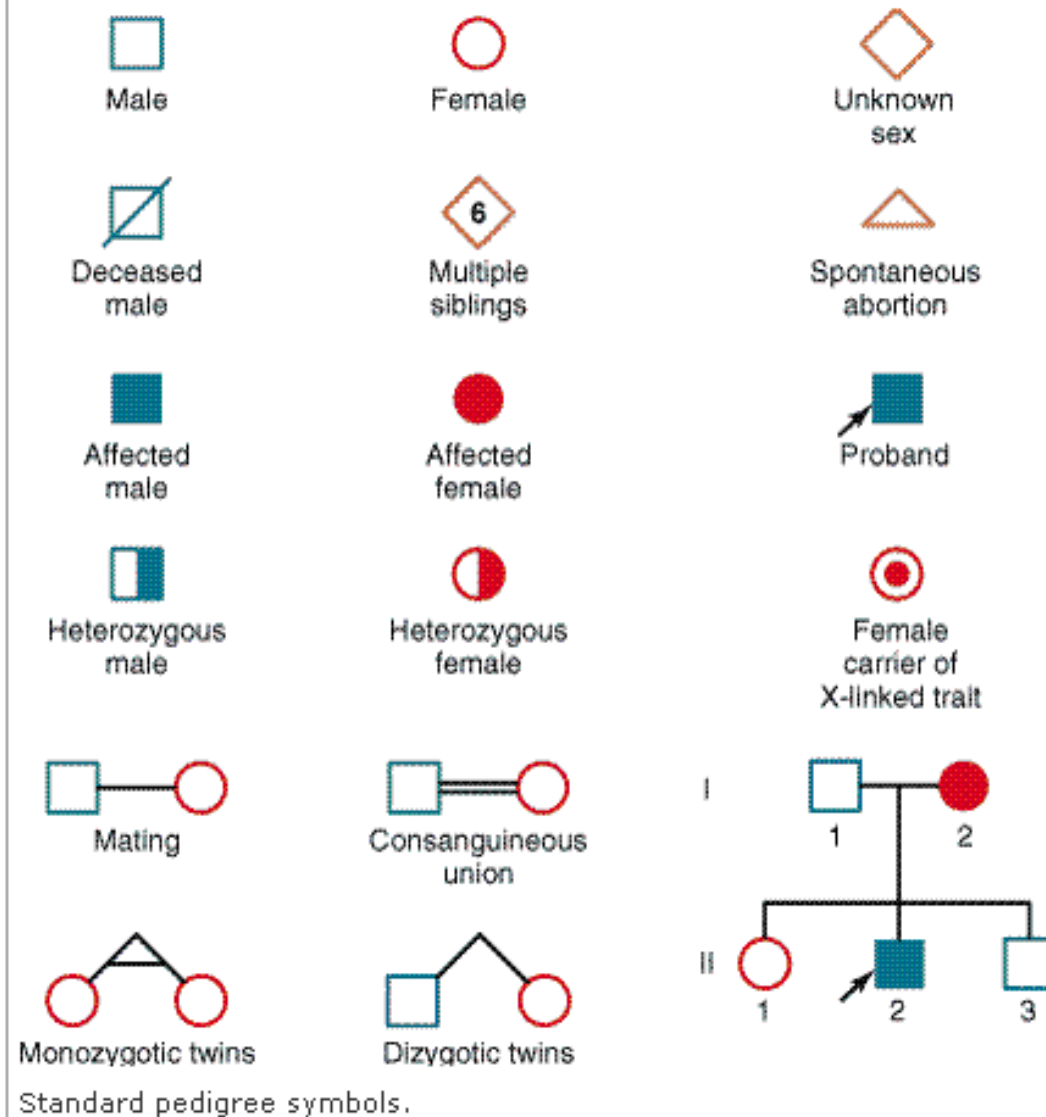
Definitions

- ▶ Hereditary
- ▶ Familial
- ▶ Congenital
 - congenital means born with
 - not all genetic diseases are congenital



Standard Pedigree Symbols

Figure 56-1



Gene

- ▶ *A functional unit that is regulated by transcription and encodes a product, either a protein or RNA*
- ▶ There are about 30,000 genes in the human genome (2% code for protein)
- ▶ A single gene can generate multiple spliced mRNA products which are translated into proteins and are subject to complex posttranslational modification



Mutations

- ▶ defined as a permanent change in the DNA
- ▶ Origin
 - germ cells – transmitted to progeny
 - somatic cells – cancer and some congenital malformations
- ▶ Types of mutation
 - **Chromosomal** mutation – structural changes within the chromosome – translocations, deletions, etc
 - **Genome** mutation – loss or gain of whole chromosomes: monosomy and trisomy
 - **Gene** mutation – alterations at the level of the gene

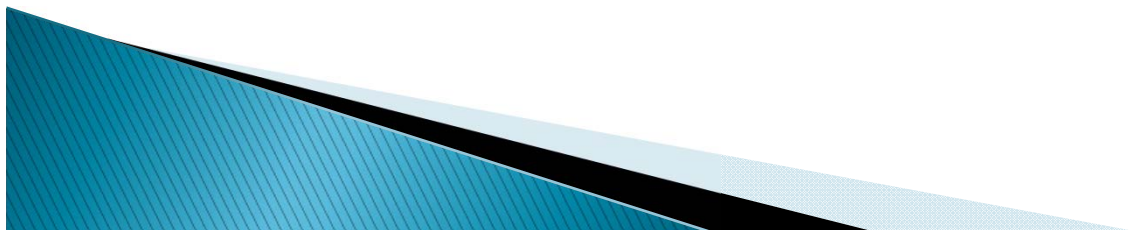


The Genetic Code

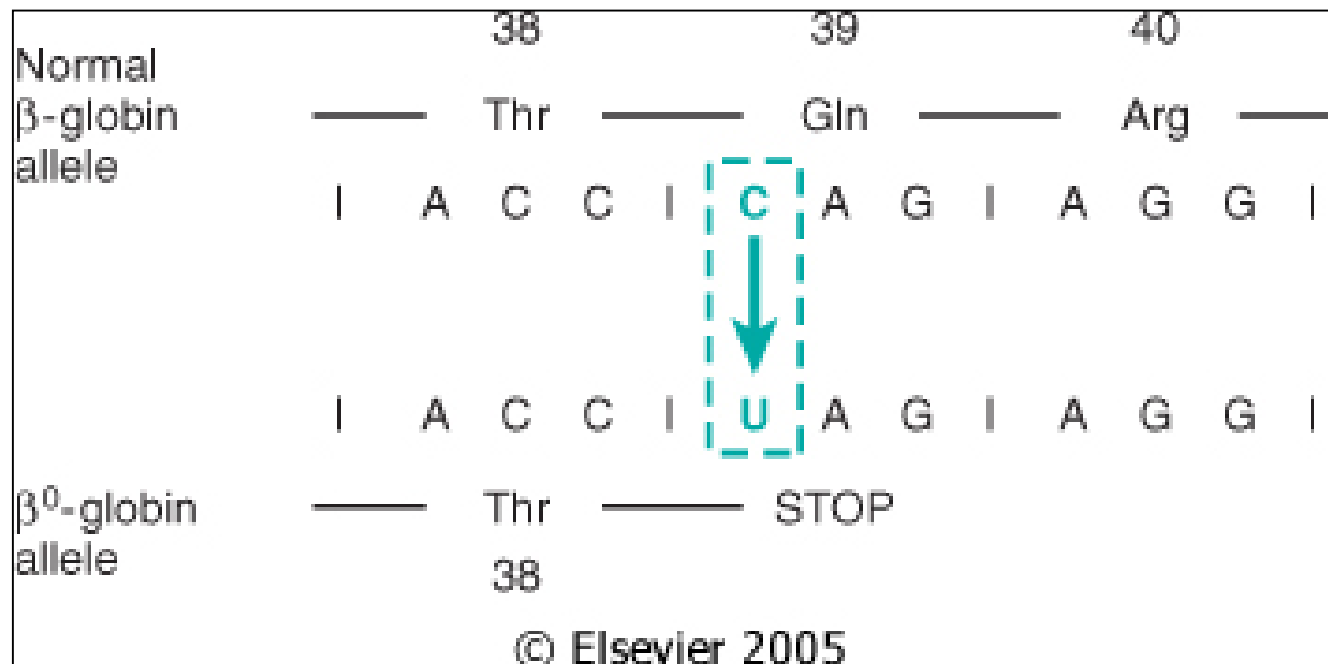
<div>UUU phenylalanine</div> <div>UUC alanine</div> <div>UUA leucine</div> <div>UUG leucine</div>	<div>UCU serine</div> <div>UCC serine</div> <div>UCA serine</div> <div>UCG serine</div>	<div>UAU tyrosine</div> <div>UAC tyrosine</div> <div>UAA stop</div> <div>UAG stop</div>	<div>UGU cysteine</div> <div>UGC cysteine</div> <div>UGA stop</div> <div>UGG tryptophan</div>
<div>CUU leucine</div> <div>CUC leucine</div> <div>CUA leucine</div> <div>CUG leucine</div>	<div>CCU proline</div> <div>CCC proline</div> <div>CCA proline</div> <div>CCG proline</div>	<div>CAU histidine</div> <div>CAC histidine</div> <div>CAA glutamine</div> <div>CAG glutamine</div>	<div>CGU arginine</div> <div>CGC arginine</div> <div>CGA arginine</div> <div>CGG arginine</div>
<div>AUU isoleucine</div> <div>AUC isoleucine</div> <div>AUA isoleucine</div> <div>AUG methionine</div>	<div>ACU threonine</div> <div>ACC threonine</div> <div>ACA threonine</div> <div>ACG threonine</div>	<div>AAU asparagine</div> <div>AAC asparagine</div> <div>AAA lysine</div> <div>AAG lysine</div>	<div>AGU serine</div> <div>AGC serine</div> <div>AGA arginine</div> <div>AGG arginine</div>
<div>GUU valine</div> <div>GUC valine</div> <div>GUA valine</div> <div>GUG valine</div>	<div>GCU alanine</div> <div>GCC alanine</div> <div>GCA alanine</div> <div>GCG alanine</div>	<div>GAU aspartic acid</div> <div>GAC aspartic acid</div> <div>GAA glutamic acid</div> <div>GAG glutamic acid</div>	<div>GGU glycine</div> <div>GGC glycine</div> <div>GGA glycine</div> <div>GGG glycine</div>

Point mutation

- ▶ Result from substitution of a single base in the DNA
- ▶ Coding portion of gene
 - **Missense**– result in substitution of one amino acid for another in the coded protein
 - conservative – function not affected
 - nonconservative – function altered
 - **Nonsense**
 - stop codon – results in truncated protein
- ▶ Noncoding portion of gene
 - promoter and enhancer regions
 - posttranslational processing – defective splicing



β^0 Thalassemia: Point Mutation Leading To Premature Chain Termination

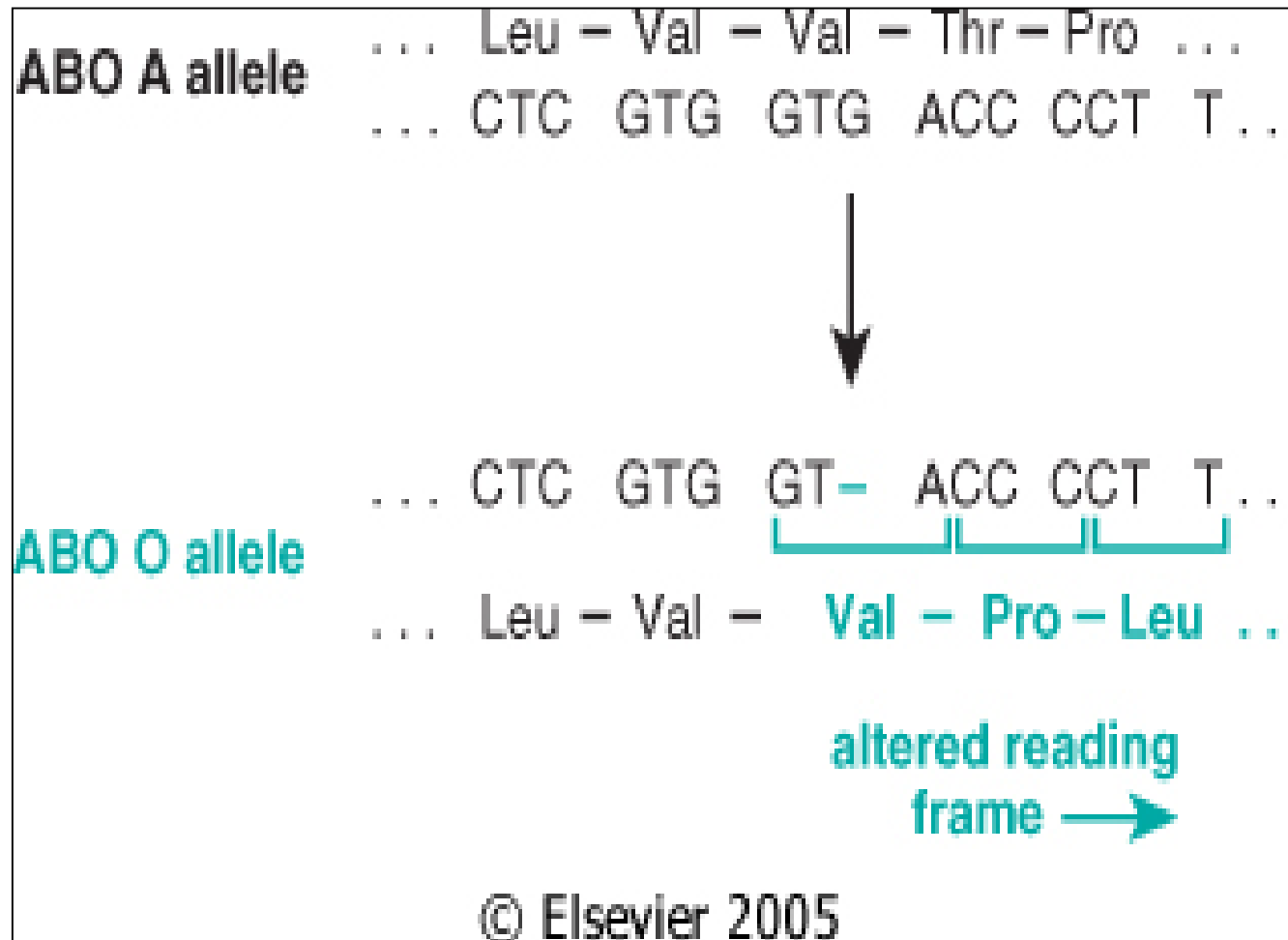


Deletions and Insertions

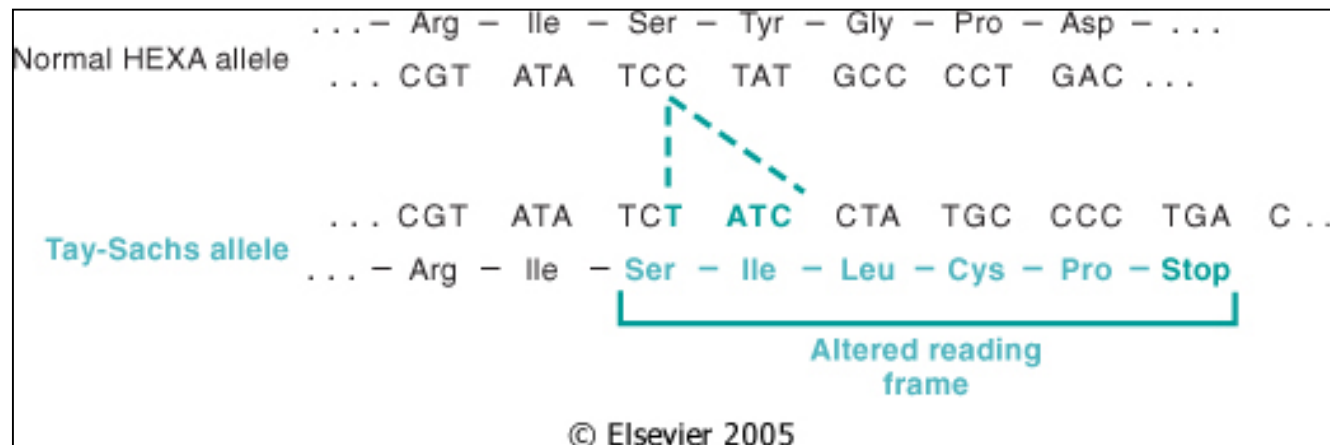
- ▶ Deletion of multiple of 3 bases
- ▶ Frameshift mutation
 - genetic code is altered distal to the mutation
 - usually leads to stop codon



Blood Group O: Single-base deletion at the ABO locus



Tay-Sachs Disease: Four-base Insertion In The Hexosaminidase A Gene



Three-base Deletion In The Common Cystic Fibrosis (CF) Allele

Normal DNA ... T ATC ATC TTT GGT GTT ..
 — Ile — Ile — Phe — Gly — Val —

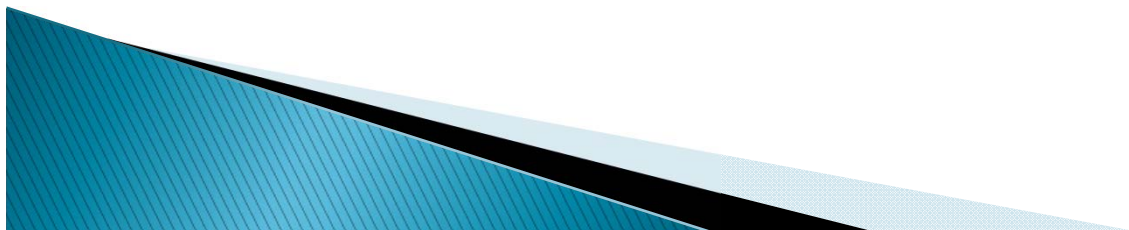
CF DNA ... T ATC AT— —T GGT GTT ..
 — Ile — Ile ————— Gly — Val —

 ΔF508

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GENE MUTATIONS

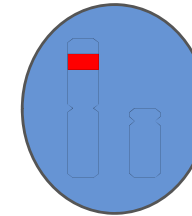
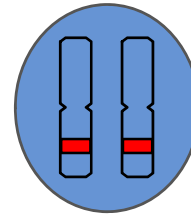
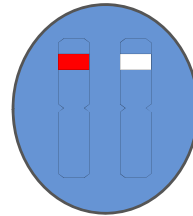
- ▶ INTERFERE with protein synthesis
- ▶ SUPPRESS transcription, DNA→RNA
- ▶ PRODUCE abnormal mRNA
- ▶ DEFECTS carried over into TRANSLATION
- ▶ ABNORMAL proteins WITHOUT impairing syntheses



Classification of genetic disorders

Single Gene Disorders

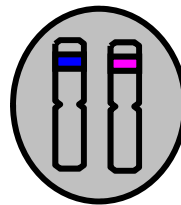
Mutations in single genes



Male

Multifactorial diseases

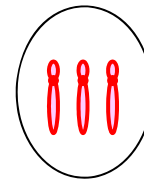
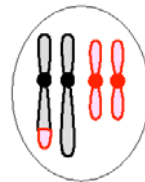
Variants in genes



+ environment

Chromosome disorders

Chromosomal imbalance

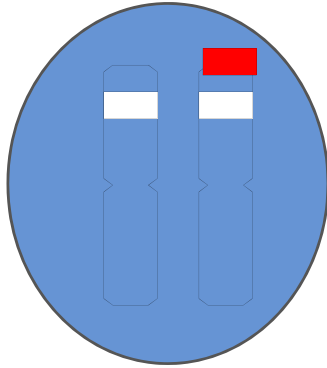


Mendelian Disorders

- ▶ All are caused by **mutations in single genes** of large effect

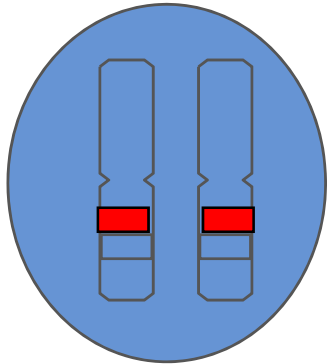


Transmission patterns of single gene disorders



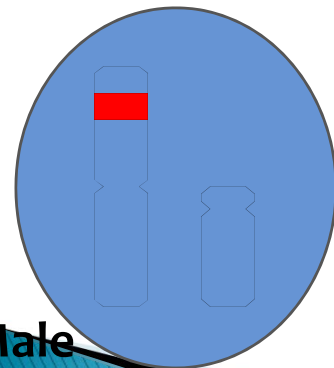
Dominant

Heterozygotes with **one copy** of the altered gene are affected



Recessive

Homozygotes with **two copies** of the altered gene are affected



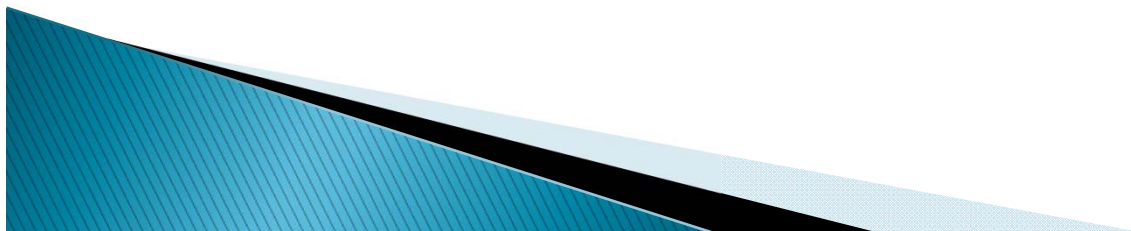
X-linked recessive

Males with **one copy** of the altered gene on the X-chromosome are affected

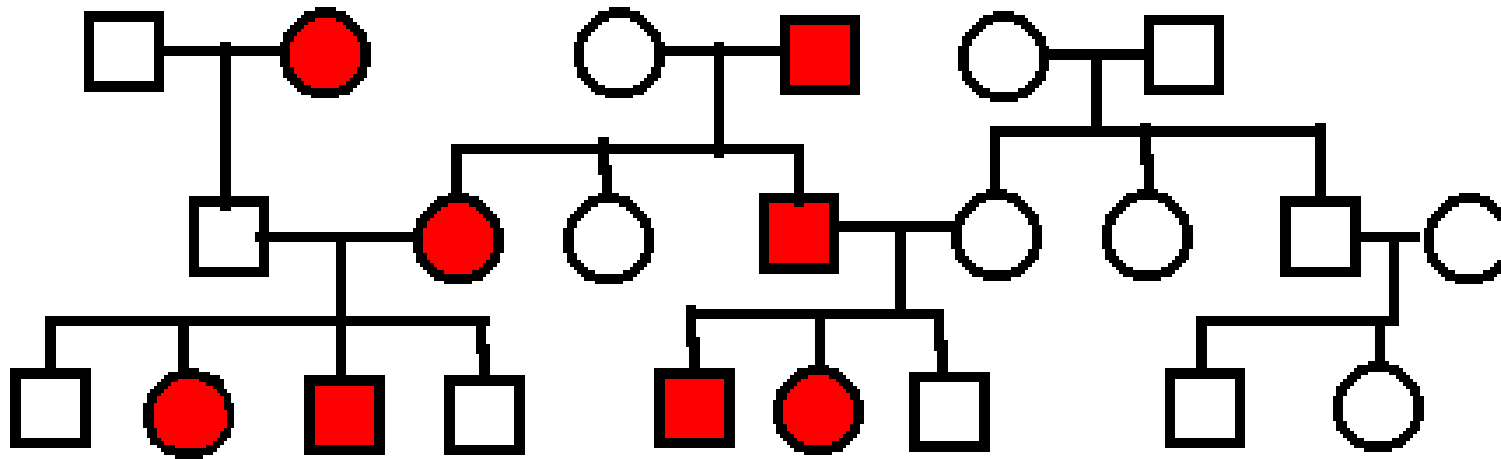
Male

Autosomal Dominant

- ▶ Disease occurs when only one allele at given gene locus is present
- ▶ **Penetrance**
 - proportion of patients who have the gene who express the trait (expressed as %)
- ▶ **Expressivity**
 - degree to which trait is expressed– e.g neurofibromatosis cases
- ▶ Role of new mutations



AUTOSOMAL DOMINANT PEDIGREE



1) BOTH SEXES INVOLVED

2) 2) GENERATIONS **NOT** SKIPPED

AUTOSOMAL DOMINANT

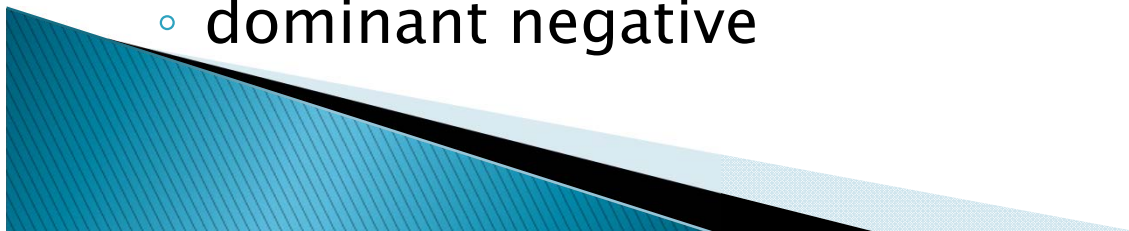
- HUNTINGTON DISEASE
- NEUROFIBROMATOSIS
- MYOTONIC DYSTROPHY
- TUBEROUS SCLEROSIS
- POLYCYSTIC KIDNEY
- HEREDITARY SPHEROCYTOSIS
- VON WILLEBRAND DISEASE
- MARFAN SYNDROME
- EHLERS–DANLOS SYNDROMES (some)
- OSTEogenesis IMPERFECTA
- ACHONDROPLASIA
- FAMILIAL HYPERCHOLESTEROLEMIA
- ACUTE INTERMITTENT PORPHYRIA



Loss of Function Mutation

Effects of Heterozygosity

- ▶ Reduced production of gene product or inactive protein.
- ▶ If Enzyme protein: not manifested usually
 - enzymes are usually present in excess
 - heterozygotes have half of normal enzyme level
- ▶ Protein involved in pathways with feedback inhibition:
 - LDL Receptor Protein in familial hypercholesteremia
- ▶ One subunit of a multimeric protein
 - e. g. collagen (trimeric molecule)
 - dominant negative



Gain of Function Mutation

- ▶ Less common than loss of function mutation
- ▶ Endow normal protein with toxic properties
- ▶ Nearly always autosomal dominant
- ▶ e. g. Huntington's disease



Autosomal Recessive

- ▶ Disease occurs only when both alleles at given gene locus are present
- ▶ Parents are usually normal
- ▶ Nearly all inborn errors of metabolism are recessive

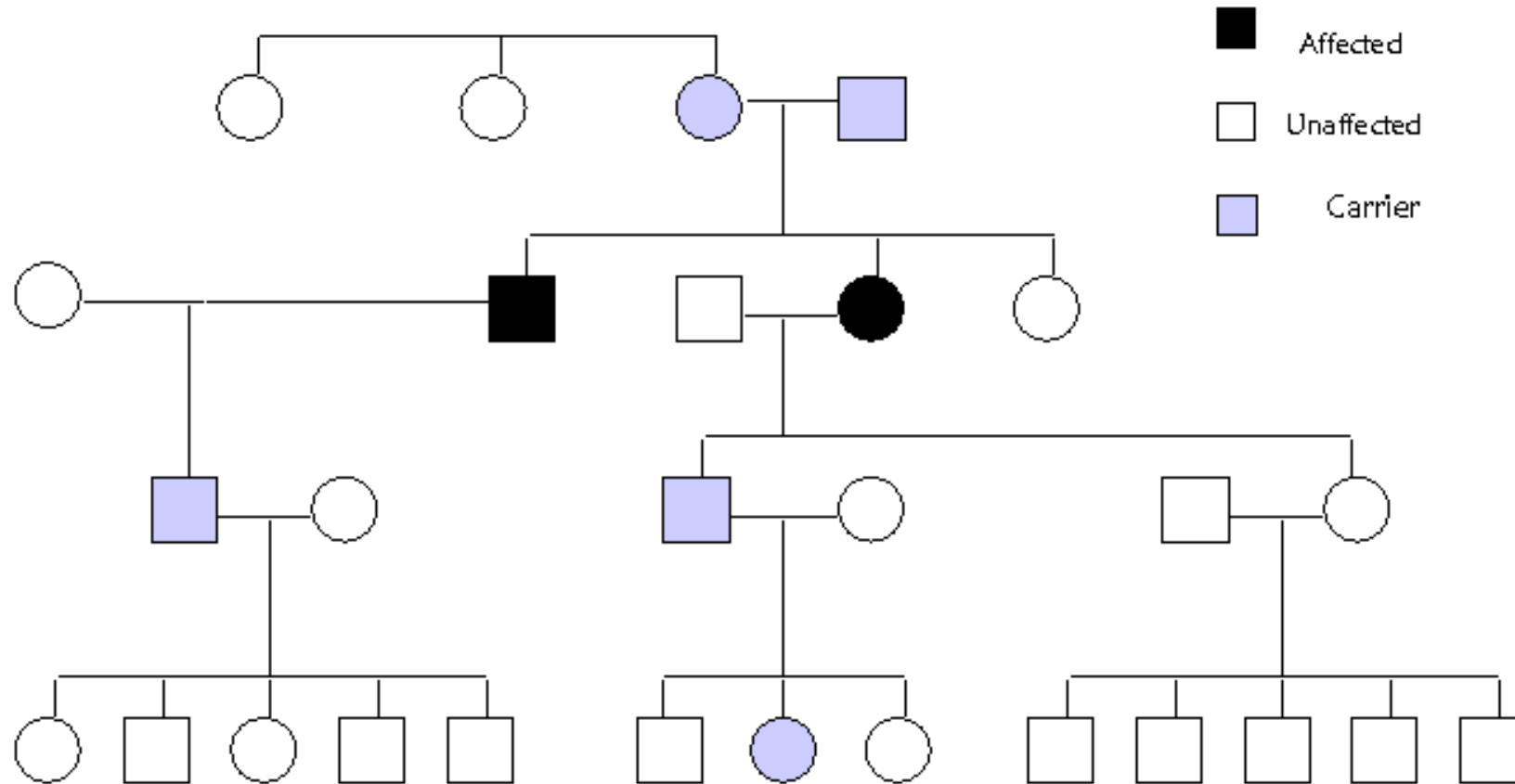


Features of Autosomal Recessive Disorders

- ▶ Expression of the disorder more uniform than with dominant diseases
- ▶ Complete penetrance is common
- ▶ Onset is frequently early in life
- ▶ New mutations may occur but are rarely detected



Autosomal Recessive



GENERATIONS SKIPPED

AUTOSOMAL RECESSIVE

- ▶ CF
- ▶ PKU
- ▶ GALACTOSEMIA
- ▶ HOMOCYSTINURIA
- ▶ LYSOSOMAL STORAGE
- ▶ A-1 ANTITRYPSIN
- ▶ WILSON DISEASE
- ▶ HEMOCHROMATOSIS
- ▶ GLYCOGEN STORAGE DISEASES

Hgb S

THALASSEMIAS

CONG. ADRENAL HYPERPLASIA

EHLERS-DANLOS (some)

ALKAPTONURIA

NEUROGENIC MUSC. ATROPHIES

FRIEDREICH ATAXIA

SPINAL MUSCULAR ATROPHY

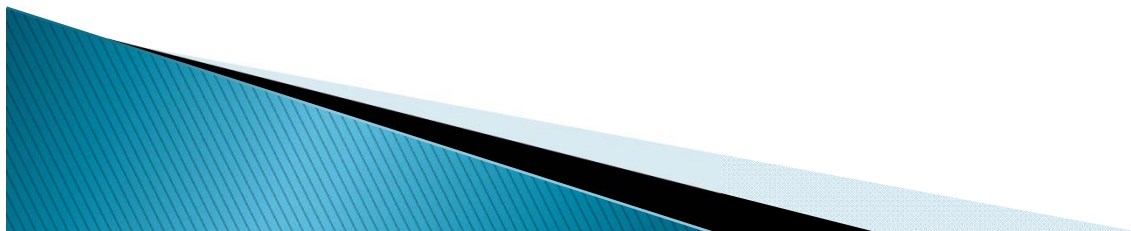
Codominance

- ▶ Both alleles of a gene pair are fully expressed in the heterozygote
- ▶ Blood group antigens
- ▶ Histocompatibility antigens



X-Linked Disorders

- ▶ Nearly all X-linked disorders are recessive
- ▶ Dominant and recessive apply only to the female – males are hemizygous
- ▶ Absence of father-son transmission
- ▶ All daughters of affected male are obligate carriers

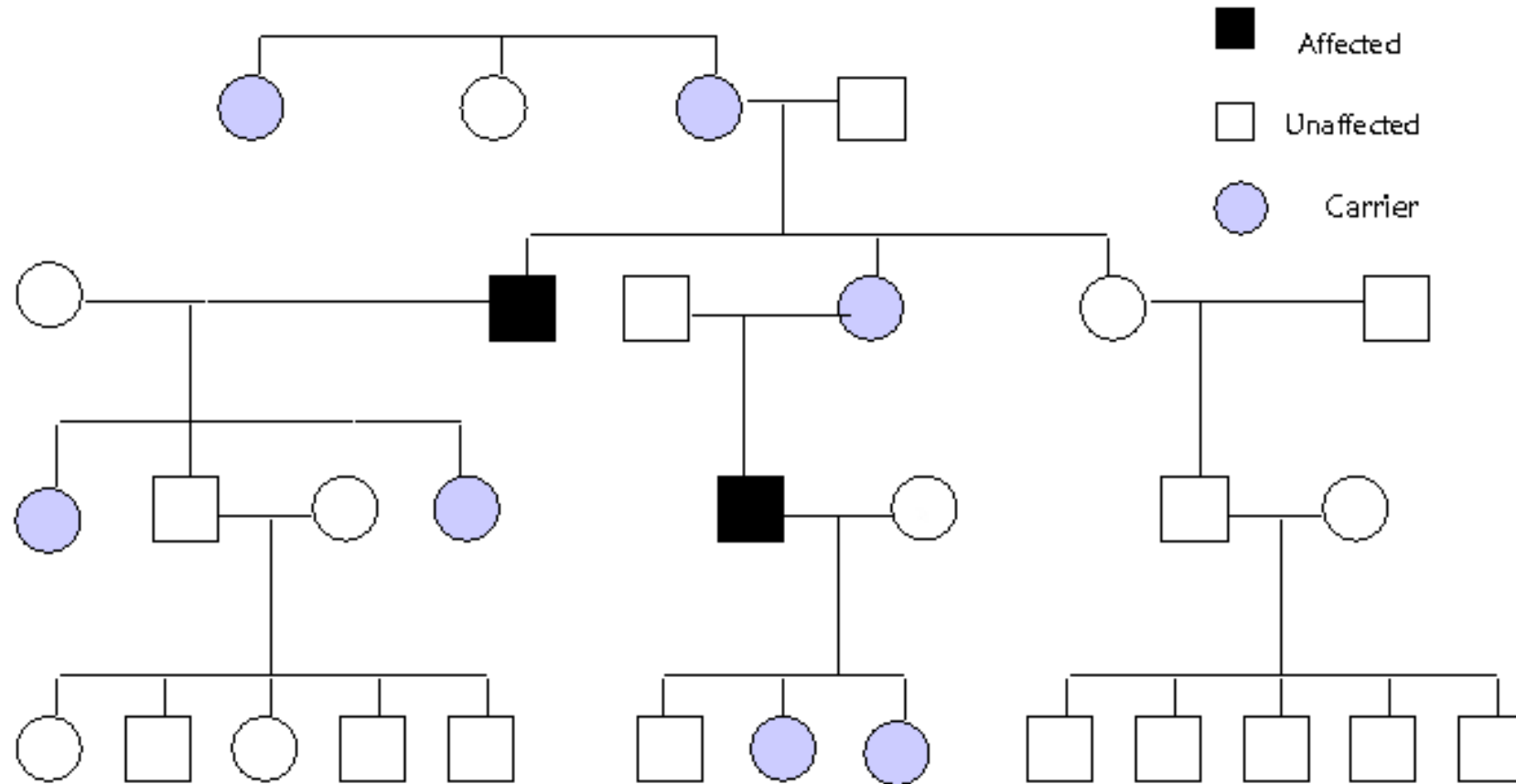


SEX (“X”) LINKED

- ▶ DUCHENNE MUSCULAR DYSTROPHY
- ▶ HEMOPHILIA , A and B
- ▶ G6PD DEFICIENCY
- ▶ AGAMMAGLOBULINEMIA
- ▶ WISKOTT–ALDRICH SYNDROME
- ▶ DIABETES INSIPIDUS
- ▶ LESCH–NYHAN SYNDROME
- ▶ FRAGILE–X SYNDROME



X-linked Recessive



1) **MALES ONLY**, sons of affected males are OK

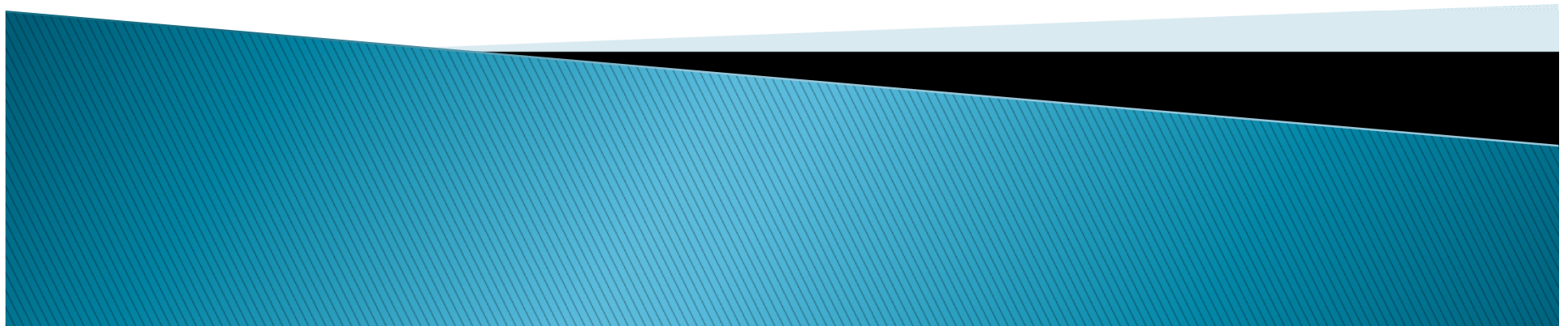
2) **GENERATION SKIPPING DOESN'T MATTER**

► Thank you



Mechanism and examples of Single gene disorders

Class 2



Mechanisms of Single Gene Disorders

1. Enzyme defects and their consequences

eg. Lysosomal storage disorders

2. Defects in membrane receptors and transport systems

eg. Familial hypercholesterolemia

3. Alterations in structure, function, or quantity of nonenzyme proteins

eg. Hemoglobinopathies, Thalassemias, Marfan's

4. Mutations resulting in unusual reactions to drugs

eg. Glucose-6-phosphate dehydrogenase (G6PD), Cytochrome P450 enzymes



Enzyme Defects and Their Consequences

- ▶ Accumulation of the substrate
- ▶ Metabolic block and decreased amount of the product (\pm lack of feedback inhibition)
- ▶ Failure to inactivate a tissue damaging substance
 - Lysosomal storage disorders
 - Lesch–Nyhan Syndrome: deficiency of HGPRT– gout
 - α_1 – antitrypsin deficiency
 - neutrophil elastase inactivation is deficient
 - unchecked activity – lung and liver damage



Defects in membrane receptors and transport systems

- Familial hypercholesterolemia
- Cystic fibrosis

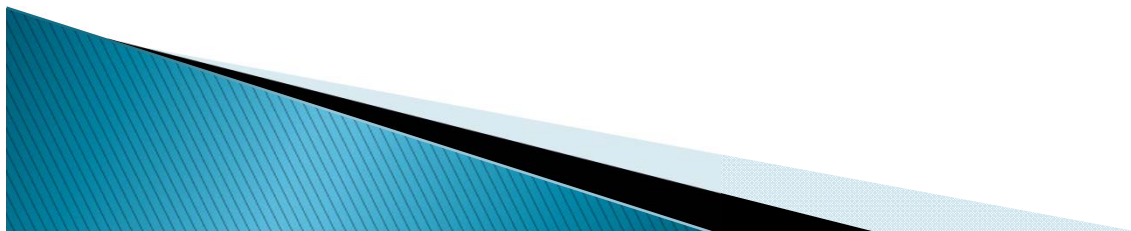
Alterations in Structure, Function or Quantity of Nonenzyme Proteins

- ▶ Hemoglobinopathies
 - sickle cell disease– abnormal β -chain
- ▶ Thalassemias
 - decreased synthesis α or β chains of hemoglobin
- ▶ Abnormal Structural Proteins
 - collagen – Ehlers–Danlos syndrome
 - elastin – Marfan's syndrome
- ▶ Muscular dystrophies

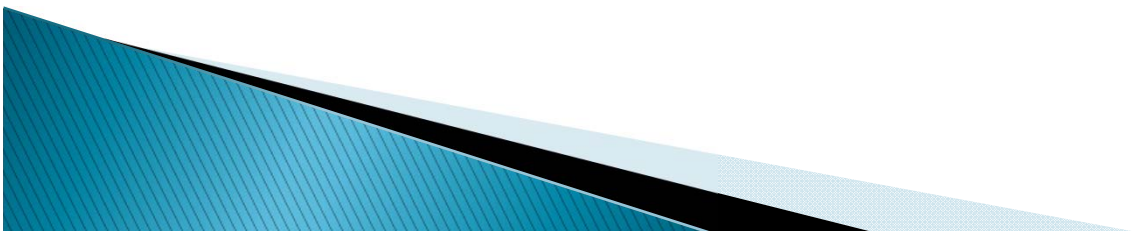


Mutations resulting in unusual reactions to drugs

- ▶ Glucose –6–phosphpate dehydrogenase (G6PD)
 - G6PD activity – protects RBCs from oxidative stress
 - drugs that block G6PD (e.g. primaquine) can cause severe hemolysis in patients who lack this enzyme
- ▶ Cytochrome P450 enzymes
 - used by the liver to metabolize many drugs
 - changes in CYP enzyme levels affect drug metabolism



- ▶ Defects in structural proteins



Marfan Syndrome

- ▶ *Marfan syndrome is a **disorder of the connective tissues** of the body, manifested principally by changes in the **skeleton, eyes, and cardiovascular system***
- ▶ Inheritance:
 - 70% to 85% – Autosomal dominant
 - Sporadic – new mutations



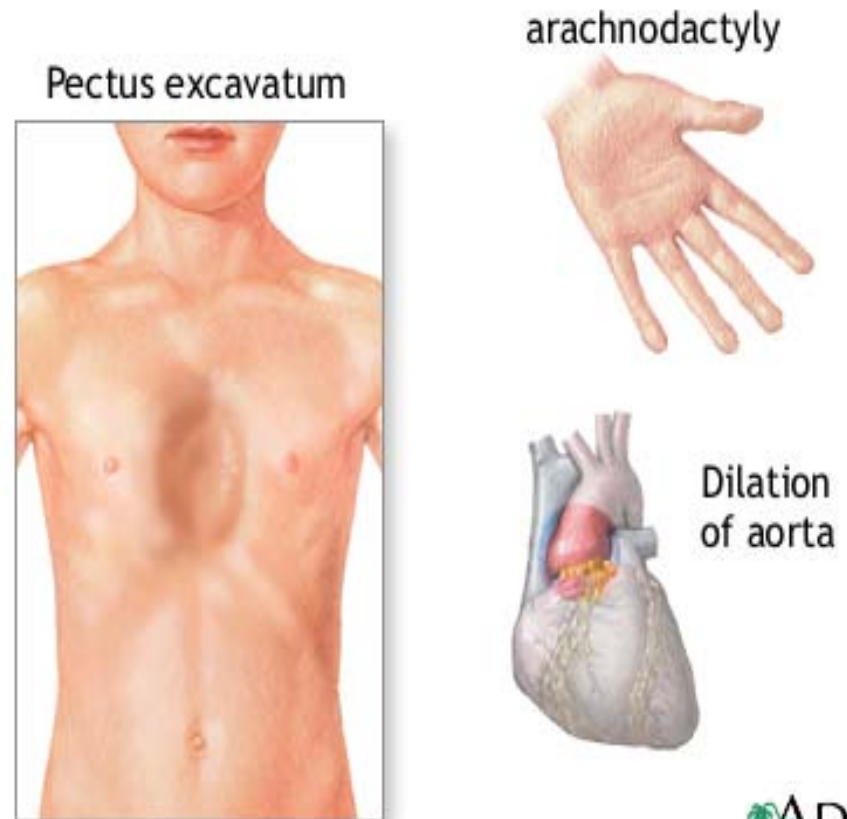
Pathogenesis

- ▶ Defect in extracellular glycoprotein *fibrillin-1*, which forms a scaffolding for deposition of elastin fibers
- ▶ mutations in FBN1 gene (chromo 15q21) – abnormal protein
- ▶ this abnormal protein disrupts assembly of microfibrils
- ▶ Microfibrils are most abundant in aorta, ligaments, and ciliary zonules(support lens)



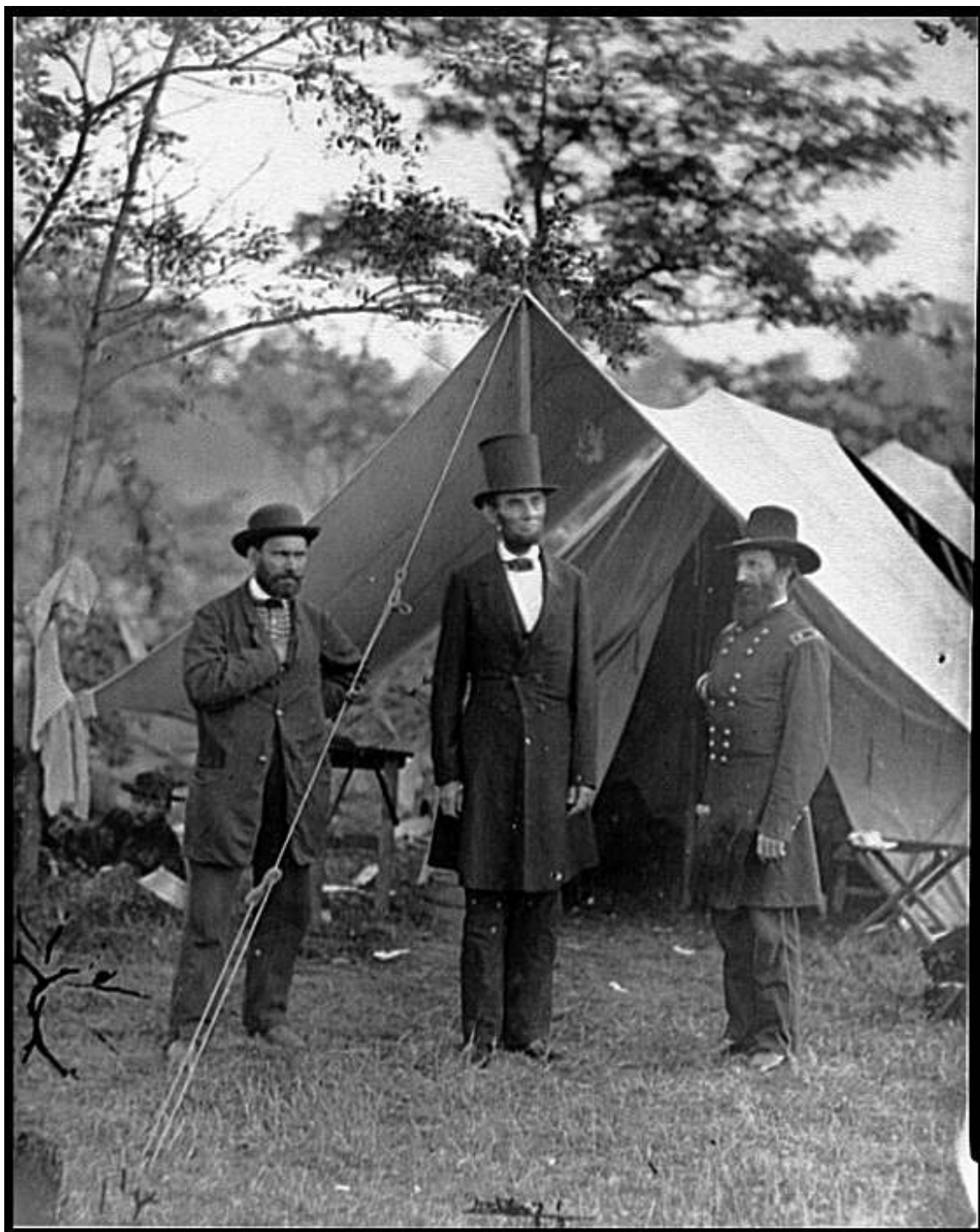
Marfan's Disease - Clinical

- **Skeletal** – most striking feature
 - Exceptionally tall
 - Pectus excavatum or pigeon chest deformity
 - Scoliosis
 - Joint laxity
 - Arachnodactyly
 - Ratio of the upper segment to the lower segment of body 2 SDs below mean



Marfans

- Cardiovascular
 - dilatation and aneurysms of ascending aorta
 - floppy mitral valve
- Ocular
 - Bilateral ectopia lentis



Marfan's Disease – Diagnosis

▶ Clinical

- skeletal changes
- ectopia lentis
- aortic aneurysm (ultrasound or x-ray)

▶ Histology

- cystic medial necrosis of aorta (autopsy)
- dissecting aortic aneurysm most common cause of death

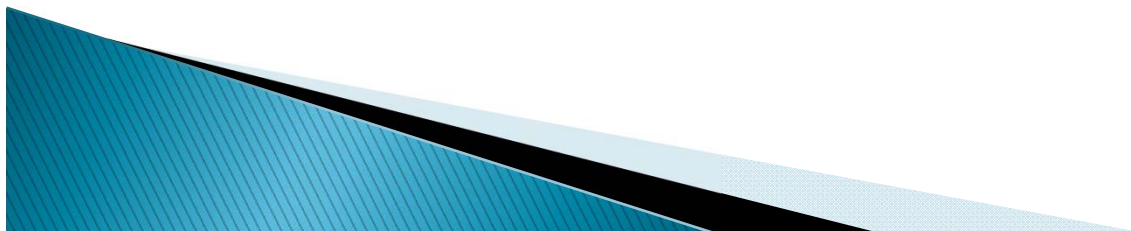
▶ Genetic and molecular

- problematic because there are 500 distinct mutations
- detection of fibrillin defects in cultured skin fibroblasts and DNA analysis of the gene by RFLP.

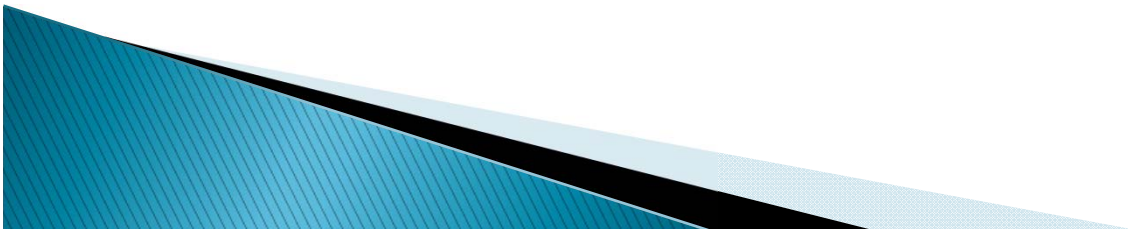


Ehler Danlos syndrome

- ▶ Defect in collagen synthesis or structure
- ▶ Type I and III collagen affected
- ▶ Tissues rich in collagen – skin, ligaments and joints
- ▶ Hyperextensible, fragile skin
- ▶ Hypermobile joints, predisposition to dislocation
- ▶ Serious internal complications



- ▶ Defects in receptor proteins



Familial Hypercholesterolemia

- ▶ Possibly the most frequent Mendelian disorder, with a gene frequency of 1:500
- ▶ Mutation of the gene encoding the low density lipoprotein (LDL) receptor
- ▶ Heterozygotes
 - 2–3 x elevation of serum cholesterol
 - tendon xanthomas and premature atherosclerosis in early adulthood
- ▶ Homozygotes
 - 5–6x elevation of serum cholesterol
 - tendon xanthomas and premature atherosclerosis develop earlier
 - may have myocardial infarction by age 20 years



Mechanism

- ▶ Syn of VLDL by liver into bloodstream.
- ▶ LPL of adipose tissue capillaries cleaves it into IDL (less TG, high chol esters content)
- ▶ 50% IDL taken up by liver by LDL receptors and recycled into VLDL
- ▶ Rest of IDL converted into LDL (chol rich)
- ▶ LDL receptors in liver n systemic take up LDL through coated pits and convert it into chol used for memb syn
- ▶ Neg feedback for more chol syn



Genetics

- ▶ Chromo 19, many mutations
- ▶ 5 classes

Class I: no syn of receptor protein

Class II: receptor protein cannot be transported from ER to golgi

Class III: LDL binding domain defective

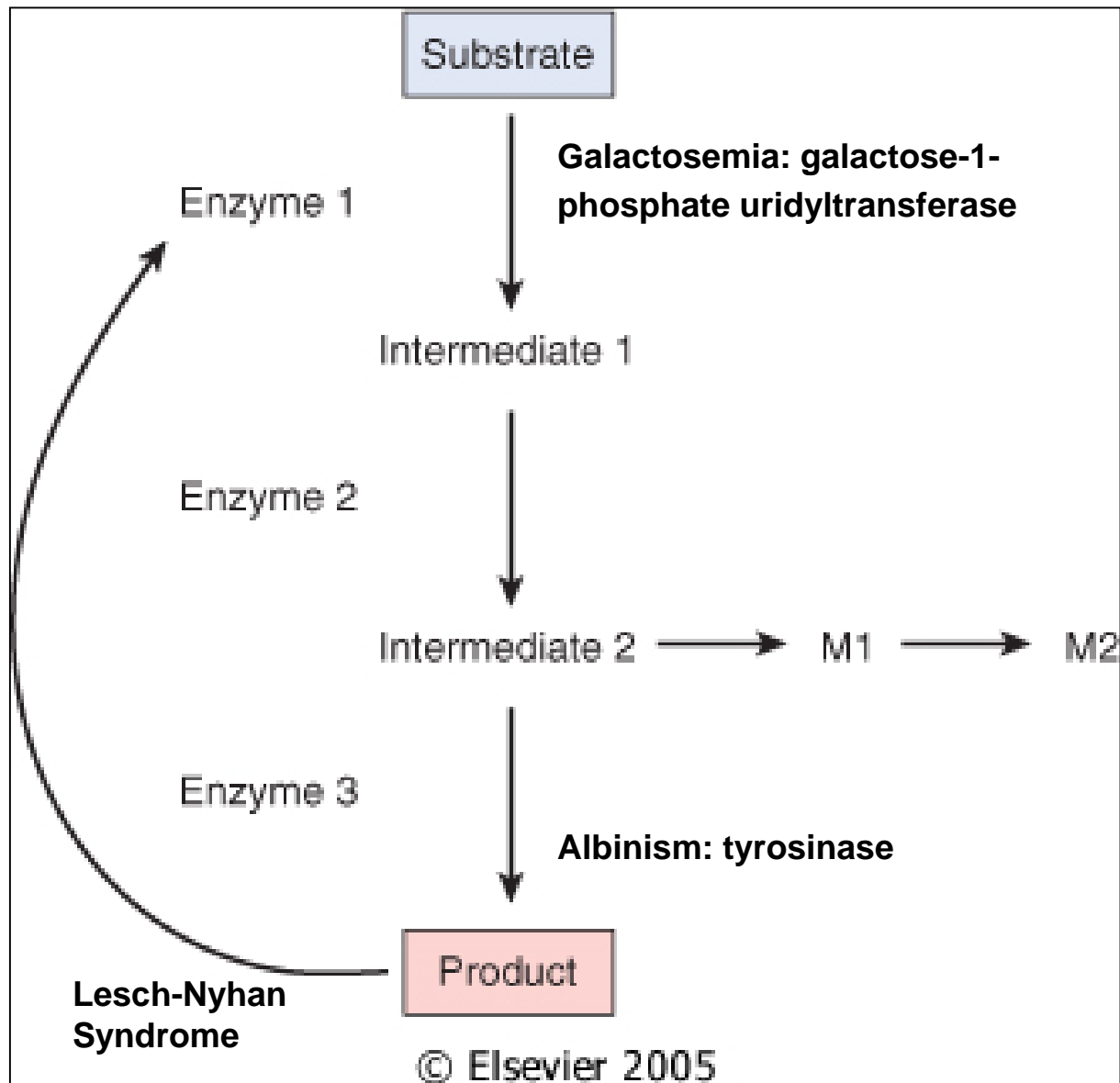
Class IV: failure to internalize the protein by coated pits

Class V: acid dependent dissociation of receptor and bound LDL fails



- ▶ Disorders associated with defect in enzymes

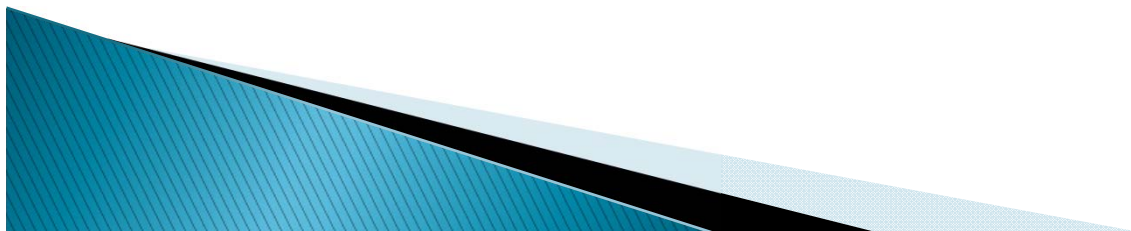




General Considerations

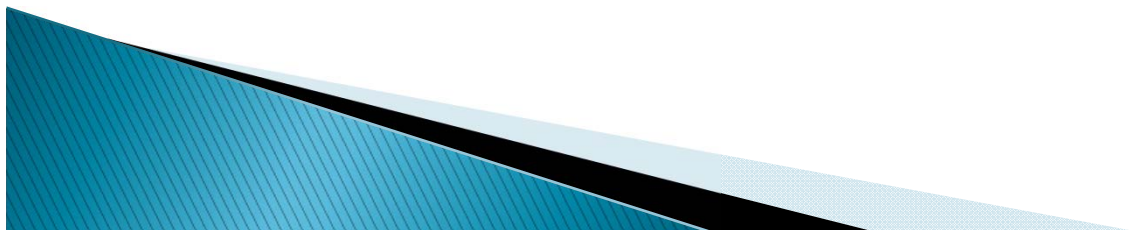
Storage Diseases

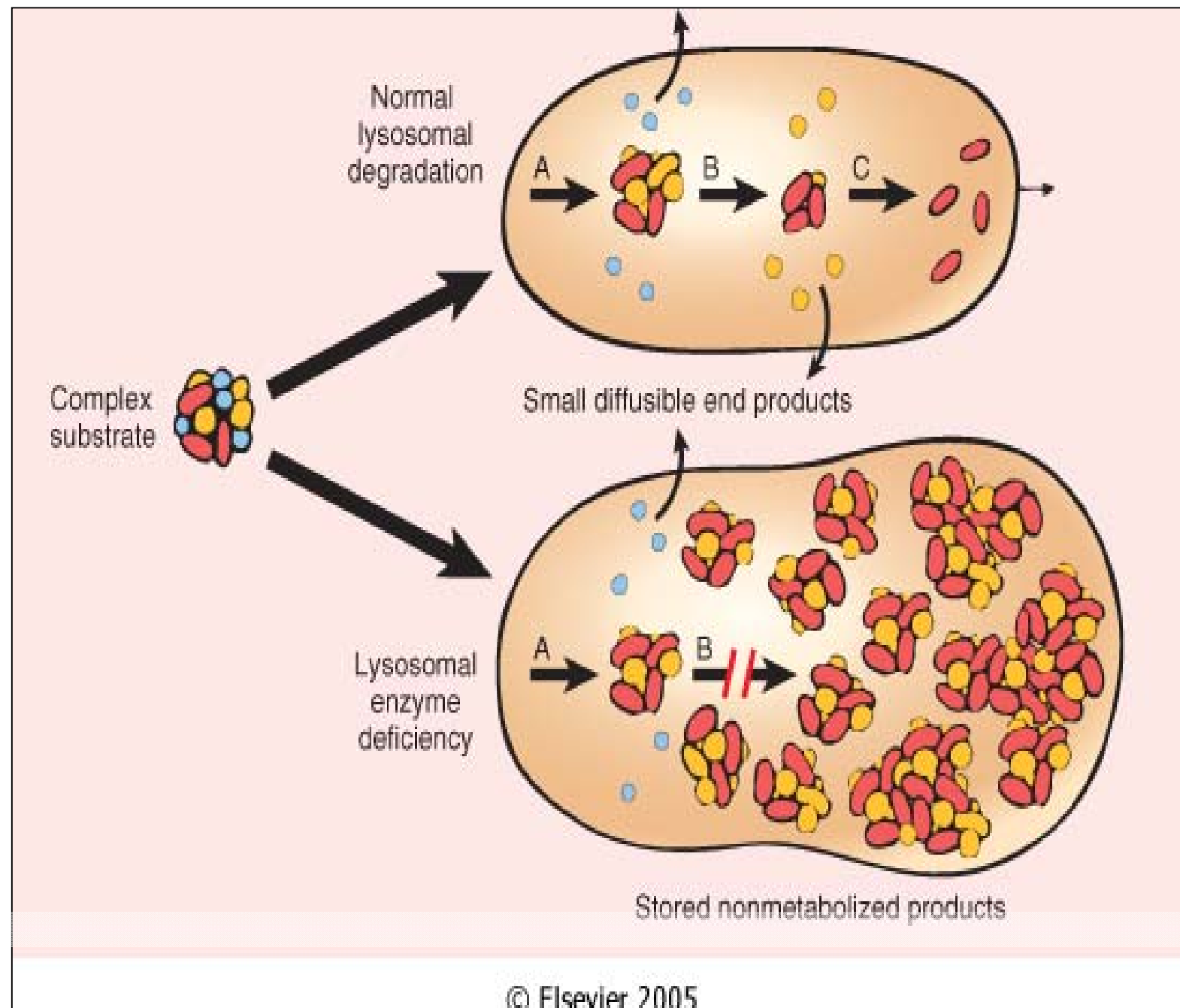
- ▶ Tissue– where most of material to be degraded is present
- ▶ Location– where degradation normally occurs
- ▶ Gaucher Disease, Type I
 - glucocerebroside in cell membranes of senescent leukocytes and erythrocytes
 - reticuloendothelial cells of spleen, bone marrow
- ▶ Tay–Sachs Disease
 - GM2 ganglioside
 - neurons of central nervous system



Lysosomal Storage Diseases

- ▶ Lysosomes contain acid hydrolases that catabolize the breakdown of complex molecules
- ▶ Lysosomes may contain
 - substances from cellular organelles (autophagy)
 - bacteria and other exogenous material (heterophagy)
- ▶ Lysosomal storage diseases result from the lack of any protein essential for their function
 - lack of lysosomal enzyme
 - dysfunctional enzyme
 - defective post-translational processing of enzyme





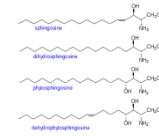
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Downloaded from: Robbins & Cotran Pathologic Basis of Disease (on 18 July 2005 09:03 PM)

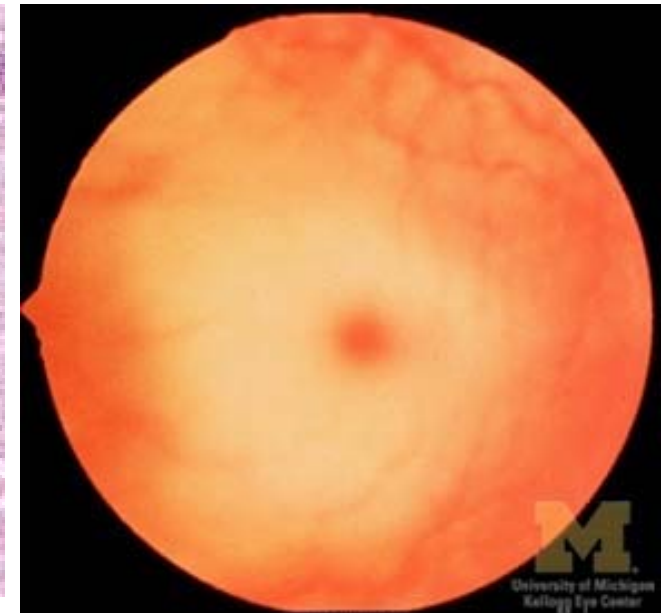
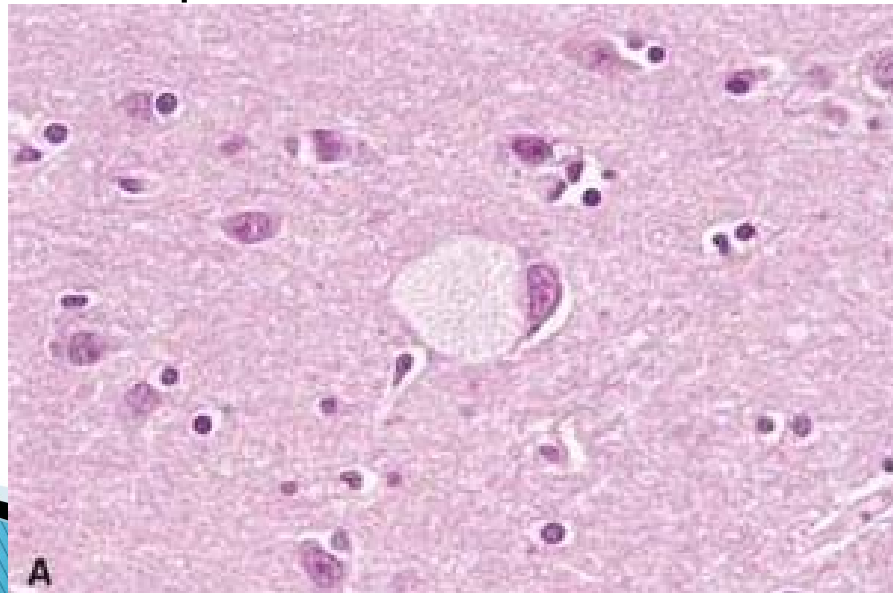
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SPHINGOLIPIDOSES



- **Tay-Sachs** mc (GM2 gangliosidosis)
- Hexosaminidase A deficiency
 - **GANGLIOSIDES** are **ACCUMULATED**
 - Ashkenazi Jews (1 / 30 are carriers)
 - CNS neurons a site of accumulation
 - Deterioration of mental and physical abilities
 - **CHERRY RED** spot in Macula



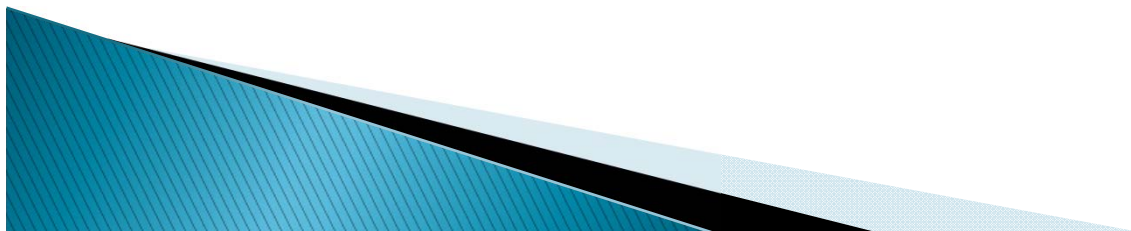
SULFATIDOSES

- ▶ MANY types, but the metachromatic leukodystrophies (CNS), Krabbe, Fabry, Gaucher, and Niemann–Pick (A and B) mc
- ▶ SULFATIDES, CEREBROSIDES, SPHINGOMYELIN are the accumulations



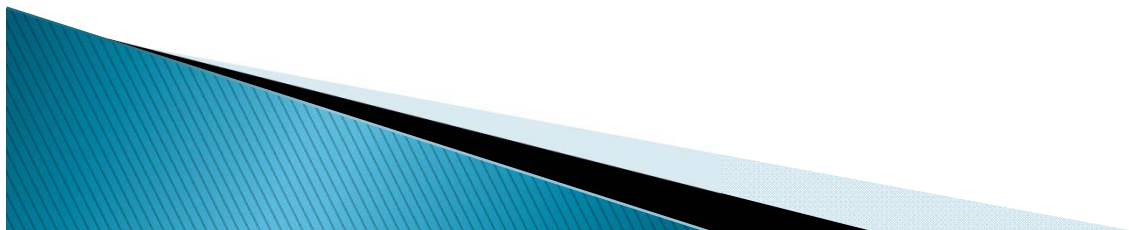
Gaucher Disease

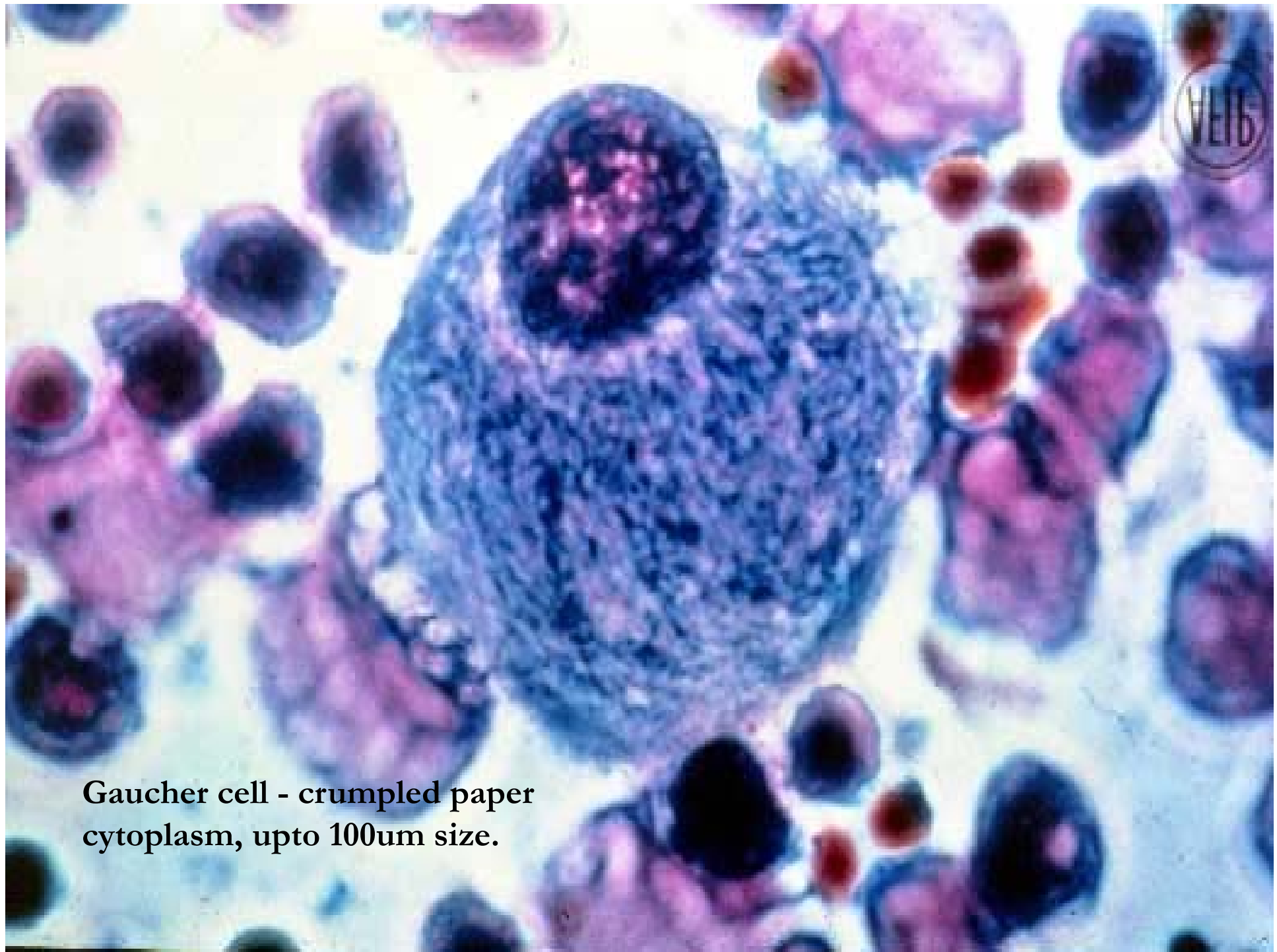
- ▶ Most common storage disease
- ▶ Autosomal recessive
- ▶ Deficient enzyme is glucocerebrosidase
 - cleaves glucose from ceramide
 - glucocerebrosides accumulate



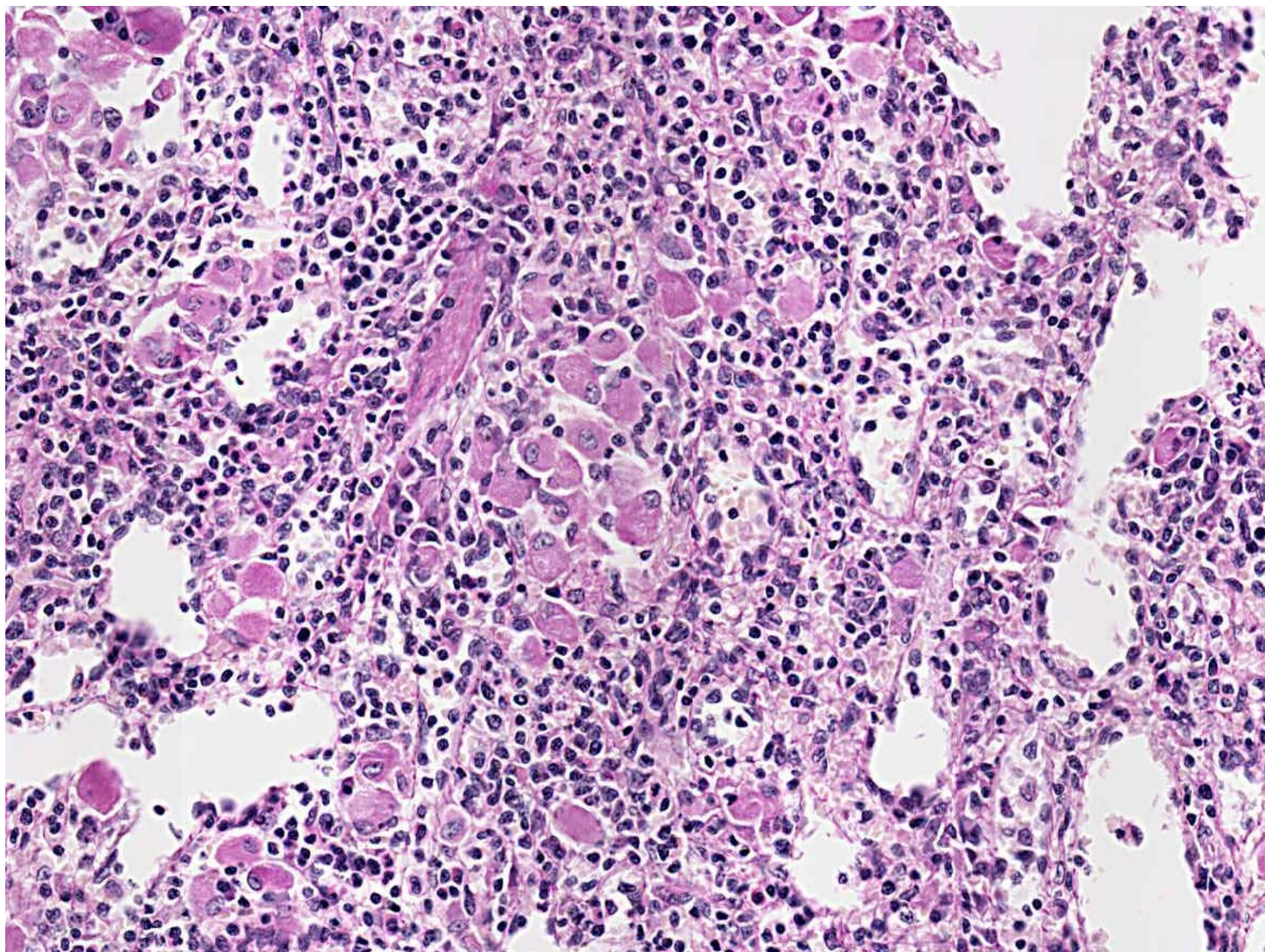
Gaucher Disease Clinical

- ▶ Glucocerebrosides are derived from catabolism of lipids in cell membranes of senescent white and red blood cells
- ▶ Accumulate in macrophages of bone marrow, liver, spleen and lymph nodes
- ▶ symptoms appear in adulthood
 - splenic enlargement
 - bone marrow involvement
 - type I disease does not cause neurological disease and is compatible with long life



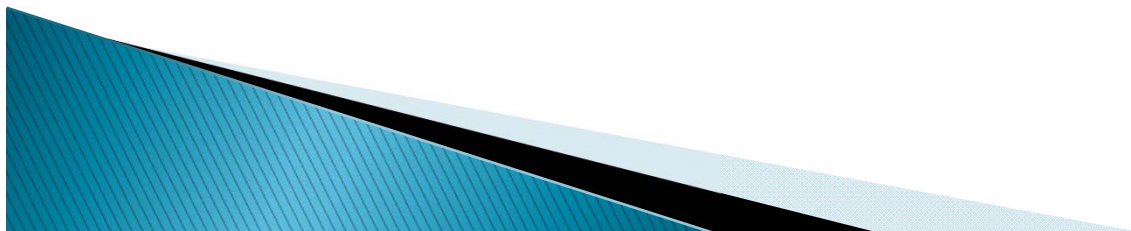


**Gaucher cell - crumpled paper
cytoplasm, upto 100um size.**



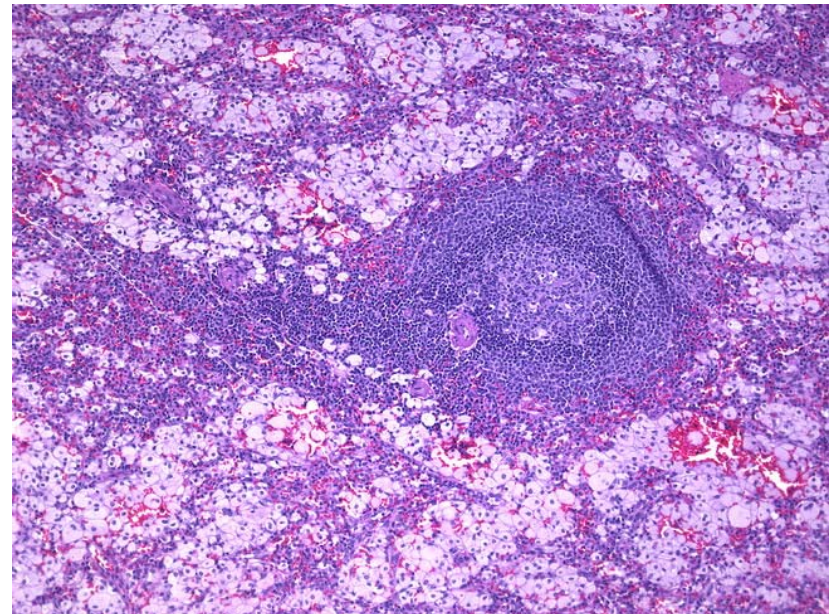
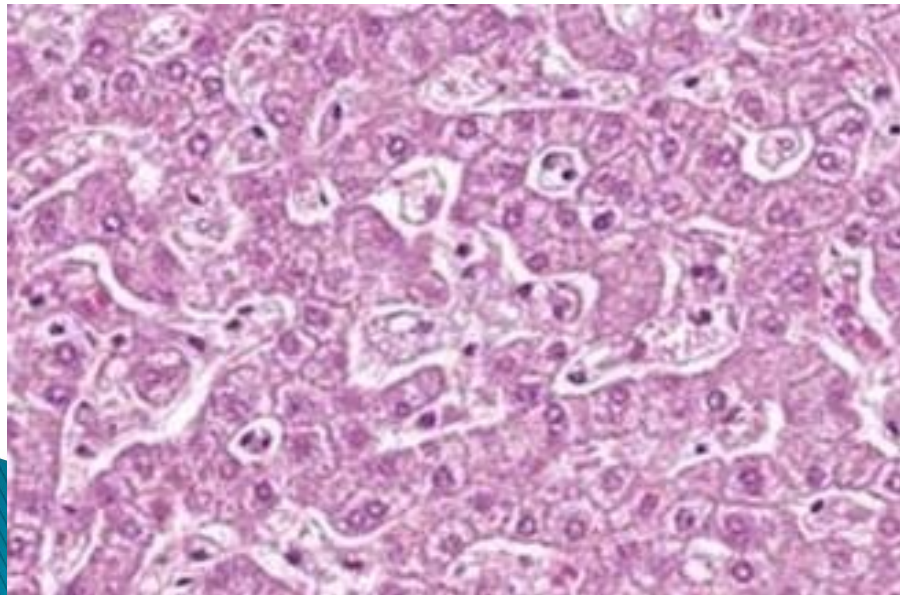
Gaucher Diagnosis

- ▶ Morphology
 - Gaucher cell is characteristic
- ▶ Glucocerebrosidase assay
 - diagnostic of homozygous disease
 - heterozygote values overlap with normal
- ▶ Genetic
 - presence of 150 alleles complicates genetic diagnosis



NIEMANN–PICK

- TYPES A, B, C
- SPHINGOMYELIN BUILDUP
- Sphingomyelinase (ASM), is the missing enzyme
- MASSIVE SPLENOMEGALY
- OFTEN FATAL in EARLY LIFE, CNS inv, ORGANOMEGALY
- Affected cells upto 90um size, foamy cytoplasm
- EM– Zebra bodies



MUCOPOLYSACCHARIDOSES



- HURLER/HUNTER, for I and II, respectively
- DERMATAN sulfate, HEPARAN sulfate buildup, respectively
 - coarse facial features
 - clouding of the cornea
 - joint stiffness
 - mental retardation
 - URINARY EXCRETION of SULFATES COMMON

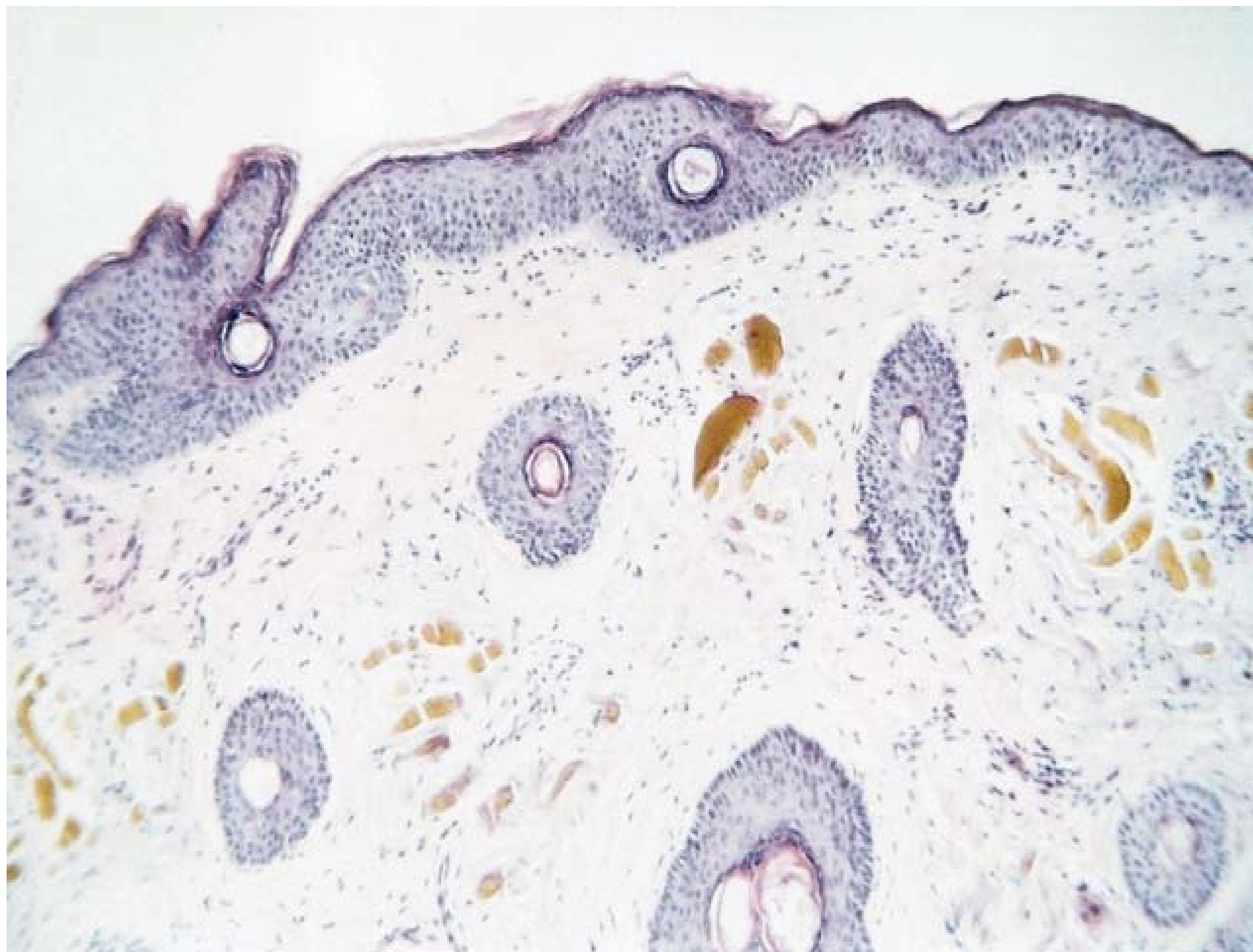
OTHER LYSOSOMAL STORAGE DIS.

- FUCOSIDOSIS
- MANNOSIDOSIS
- ASPARTYLGLYCOSAMINURIA
- WOLMAN (CHOL., TRIGLYCERIDES)
- ACID PHOSPHATE DEFICIENCY (PHOS. ESTERS)

ALKAPTONURIA

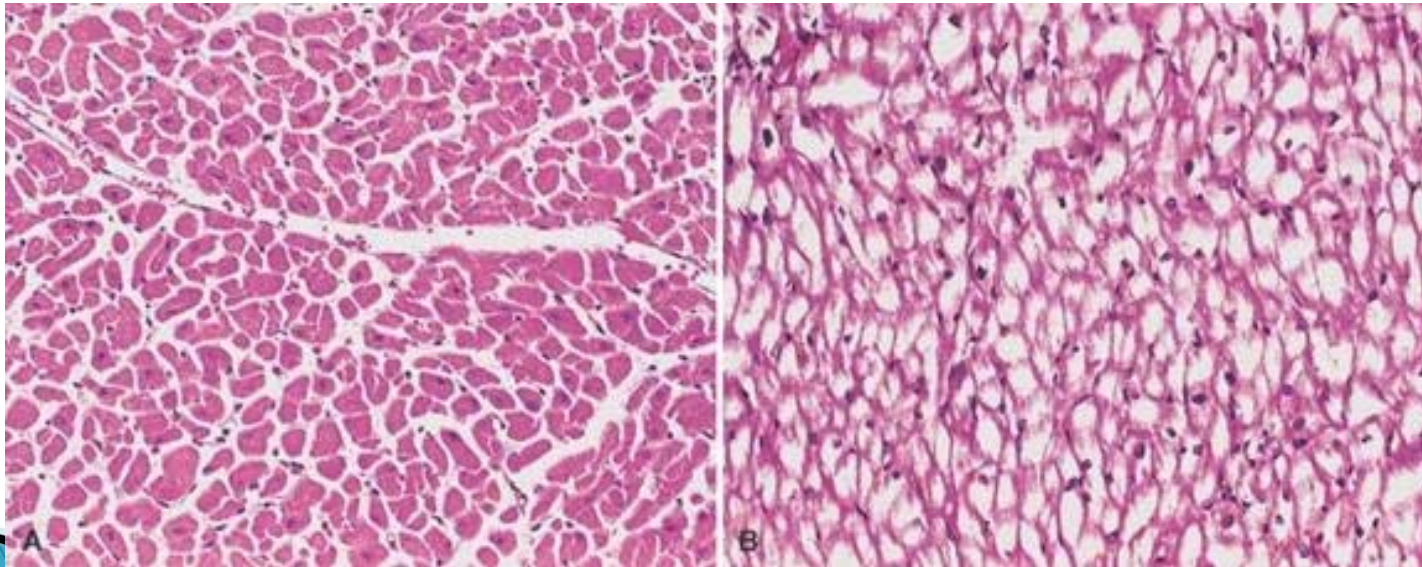
- NOT a LYSOSOMAL ENZYME DISEASE
- FIRST inborn error of metabolism TO BE DESCRIBED
- HOMOGENETISIC ACID accumulates
- HOMOGENETISIC ACID OXIDASE
 - BLACK URINE
 - BLACK NAILS (OCHRONOSIS), SKIN
 - BLACK JOINT CARTILAGE (SEVERE ARTHRITIS)



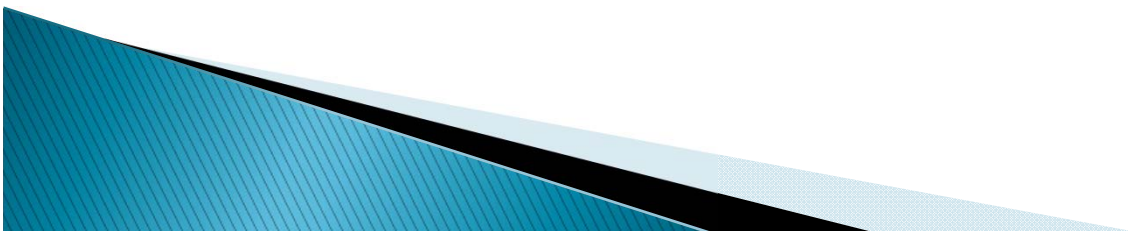


GLYCOGEN STORAGE DISEASES

- MANY TYPES (at least 13)
- Type 2 (Pompe), von Gierke, McArdle, mc
- Storage sites: **Liver**, Striated Muscle (Skel + Ht)



- ▶ Defects in proteins that regulate cell growth



Neurofibromatosis

- ▶ Example of a defect in a protein affecting cell growth – Tumor suppressor gene
- ▶ Autosomal dominant
- ▶ Type I
 - relatively common, 1 in 3000
 - 50% of cases lack positive family history and are new mutations
 - penetrance is 100%, but expresivity is very variable
- ▶ Type II
 - less common and not discussed further here



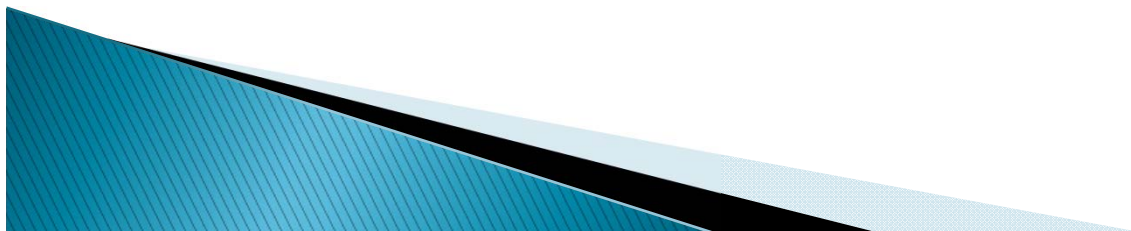
NF-1 Gene

- ▶ Gene for neurofibromatosis type 1 (NF1)
- ▶ Chromosome 17q11.2
- ▶ Encodes for a protein (neurofibromin) which down-regulates the RAS signal transduction pathway
- ▶ NF-1 belongs to a family of tumor suppressor genes



NF-1 Characteristics

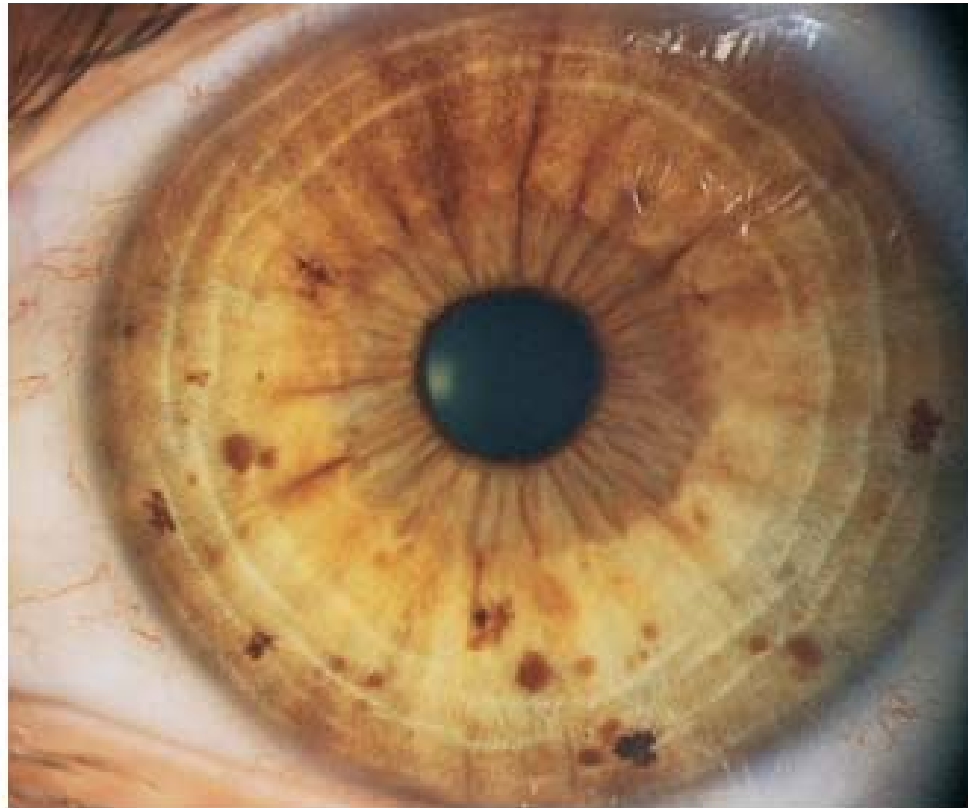
- ▶ Multiple neurofibromas dispersed anywhere in the body
 - cutaneous
 - subcutaneous
 - plexiform – diagnostic for NF-1
- ▶ Multiple pigmented skin lesions, including café au lait spots
- ▶ Pigmented iris nodules called Lisch nodules



Café Au Lait Spots



Lisch Nodules



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Plexiform Neurofibroma



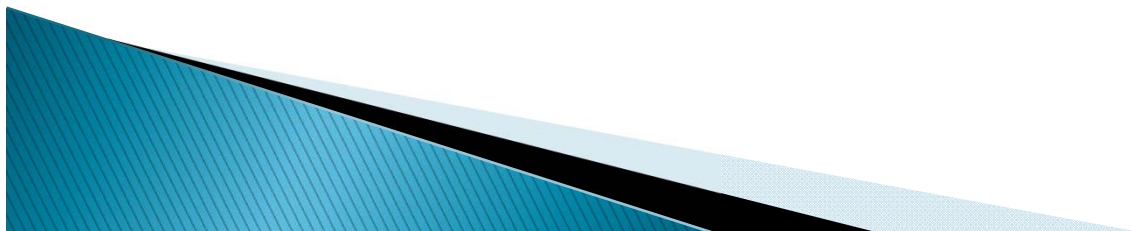
Diagnostic Criteria for Neurofibromatosis Type 1

Table 2. Diagnostic Criteria for Neurofibromatosis Type 1.

Criterion (at least two for diagnosis)	Notes
Six or more café-au-lait macules	>5 mm before puberty >15 mm after puberty
Skin-fold freckles	Groin, axilla, neck base
Neurofibromas	Two or more neurofibromas or one plexiform
Skeletal dysplasia	Orbital or tibial
Lisch nodules	Two or more iris hamartomas
Optic glioma	Detected by MRI
Family history	First-degree relative

NF-1 Genetic Testing

- ▶ Until recently no tests were available
 - NF-1 is a large gene with 60 exons
 - gene has high mutation rate
 - hundreds of mutations have been reported and almost no two families share the same mutation
- ▶ Specialized methods are now available
 - sequencing of entire gene
 - protein truncation analysis – neurofibromin

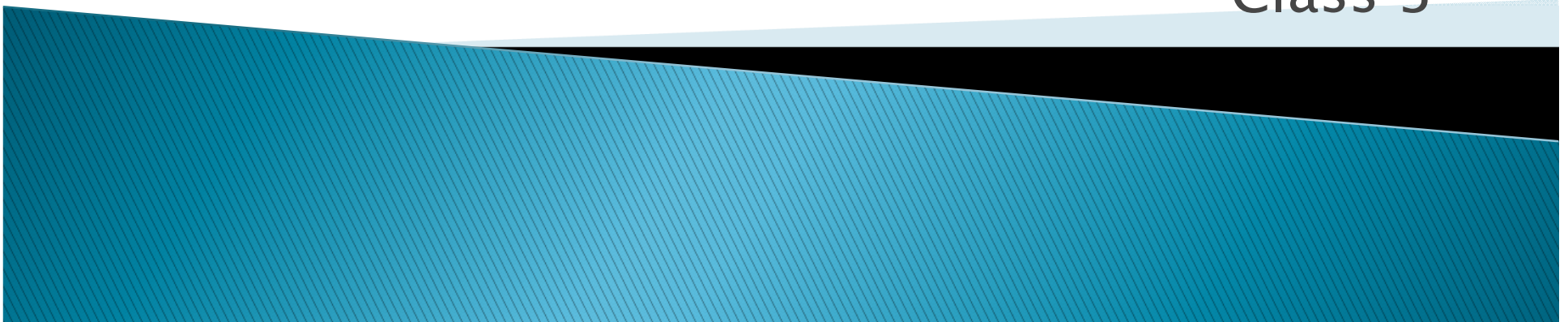


► Thank you



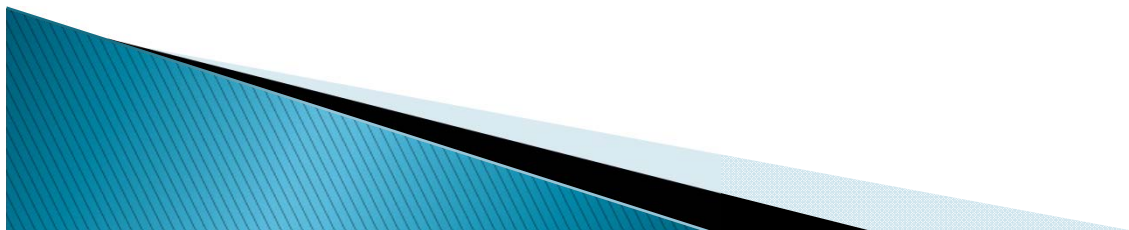
Multifactorial inheritance and Chromosomal disorders

Class 3



MULTIFACTORIAL INHERITANCE

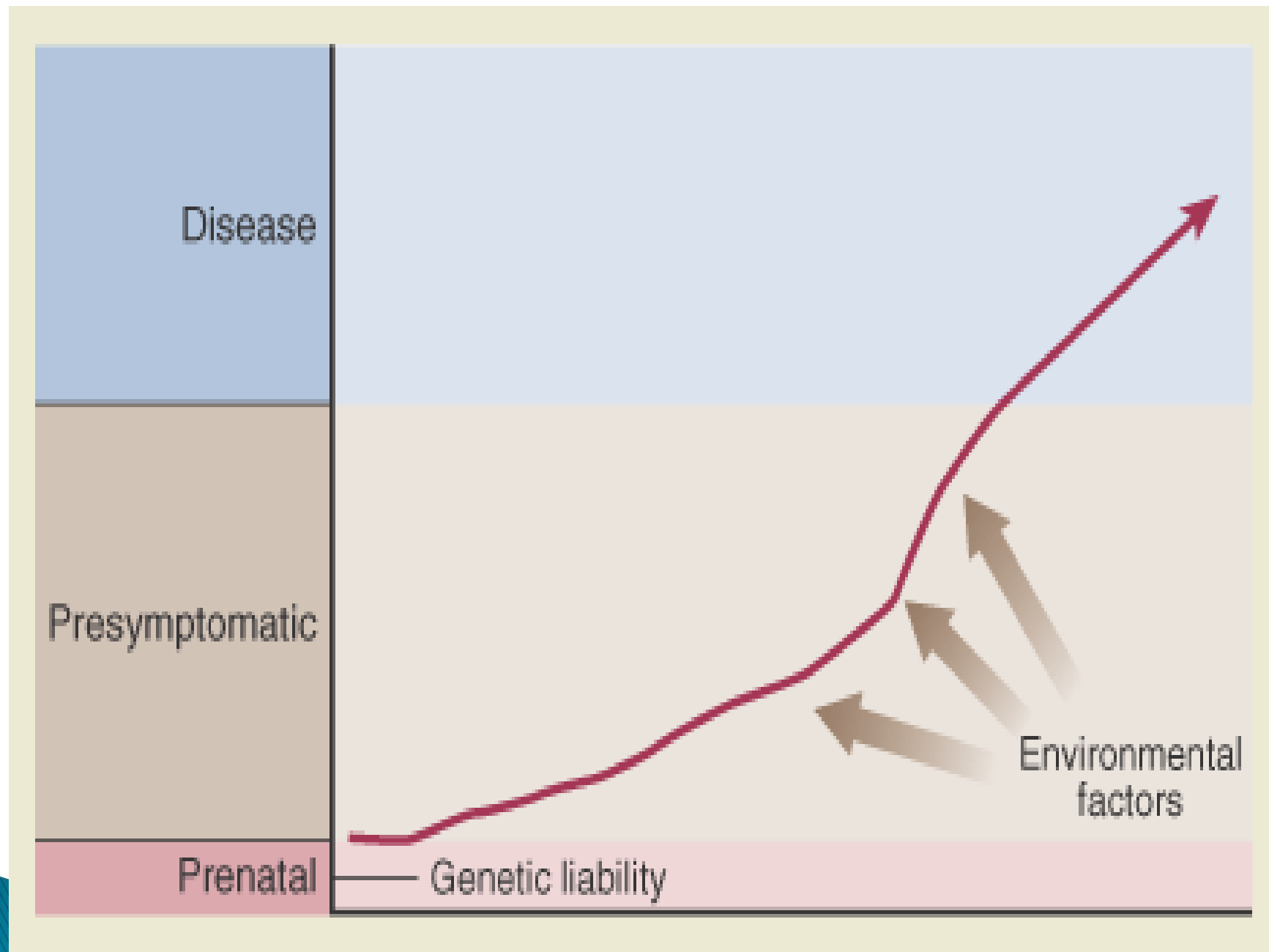
- ▶ Multi-“FACTORIAL”, not just multi-GENIC “SOIL” theory
- ▶ Common phenotypic expressions governed by “multifactorial” inheritance
 - Hair color
 - Eye color
 - Skin color
 - Height
 - Intelligence
 - Diabetes, type II



FEATURES of multifactorial inheritance

- ▶ Expression determined by NUMBER of genes
- ▶ Overall 5% chance of 1st degree relatives having it
- ▶ Identical twins >>>5%, but WAY less than 100%



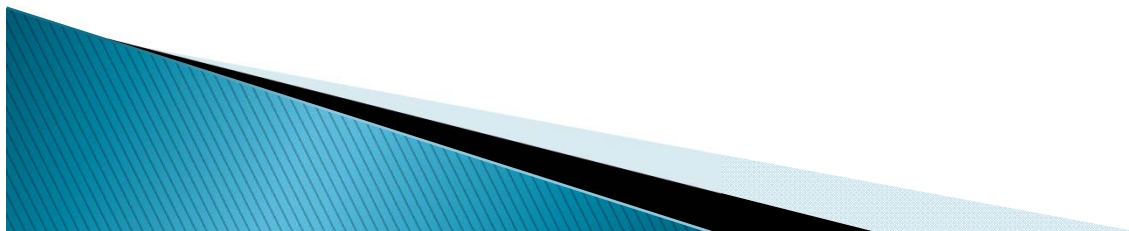


“MULTIFACTORIAL” DISORDERS

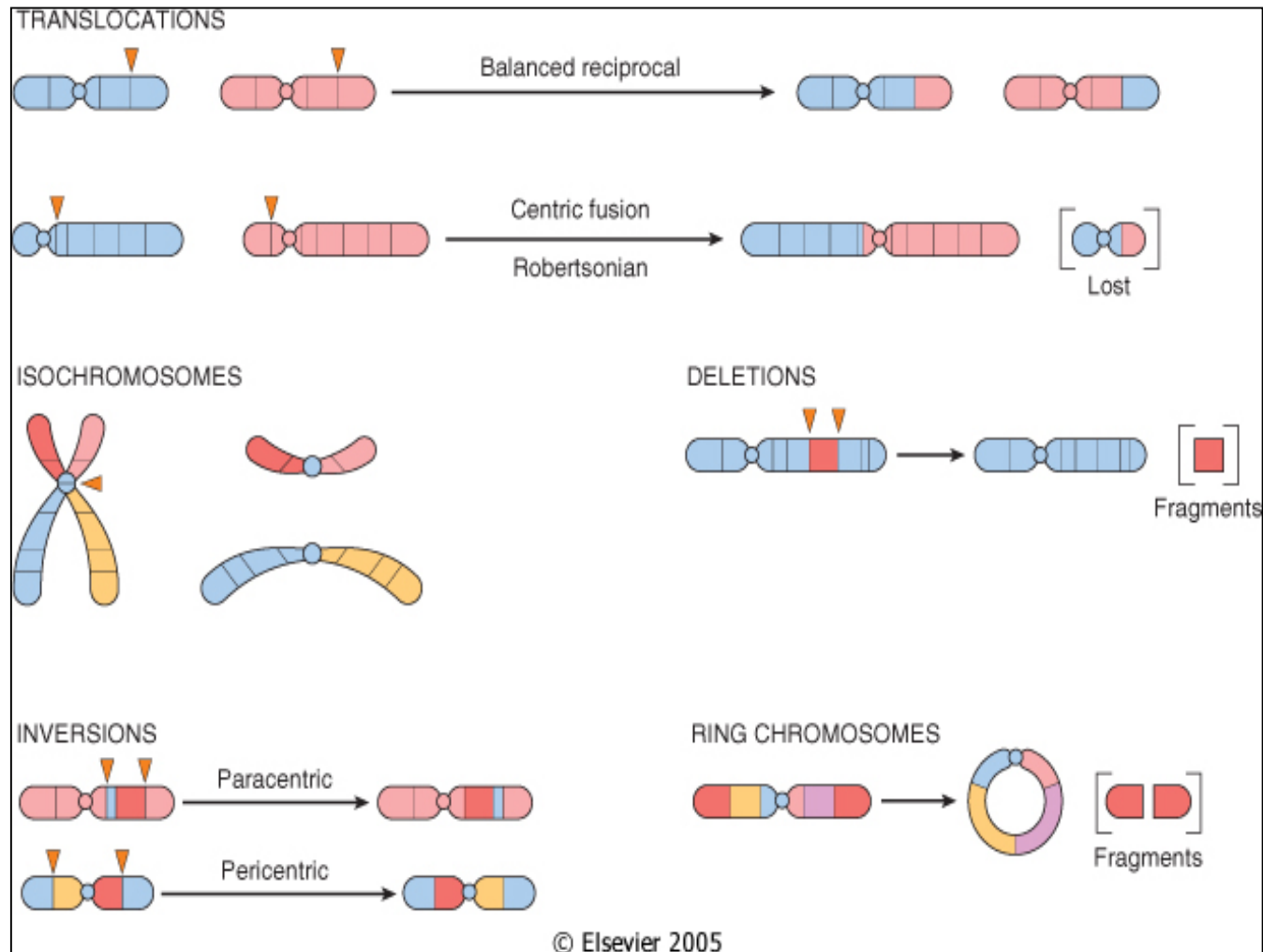
- ▶ Cleft lip, palate
 - ▶ Congenital heart disease
 - ▶ Coronary heart disease
 - ▶ Hypertension
 - ▶ Gout
 - ▶ Diabetes
 - ▶ Pyloric stenosis
 - ▶ MANY, MANY, MANY, MANY
MORE.....
- 

Cytogenetic Disorders

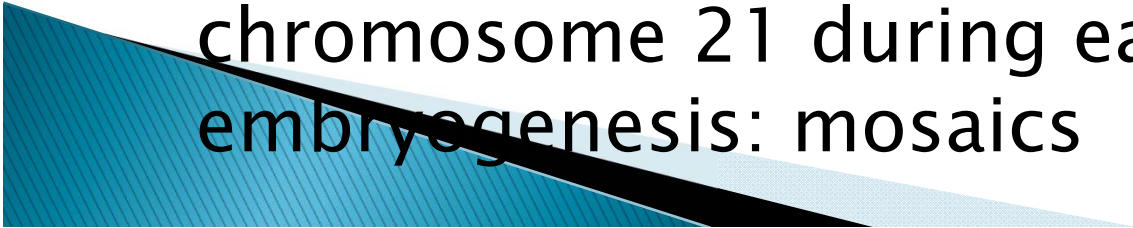
- ▶ Chromosome mutation – structural changes within the chromosome
 - deletion
 - inversion
 - translocation
- ▶ Genome mutation – loss or gain of whole chromosomes: monosomy and trisomy
 - sex chromosomes
 - autosomes



Types Of Chromosomal Rearrangements.



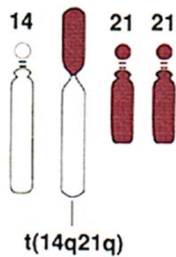
Trisomy 21–Down Syndrome

- ▶ Most common chromosomal disorder
 - ▶ Affects 1 in 750 newborns overall, but is related to maternal age
 - 1 in 1550 live births of mothers > 20 years
 - 1 in 25 live births of mothers > 45 years
 - ▶ Usually results from meiotic nondisjunction of chromosome 21
 - ▶ 4% result from Robertsonian translocation of chromosome 21 to another chromosome
 - ▶ 1% result from mitotic nondisjunction of chromosome 21 during early embryogenesis: mosaics
- 

Three different patterns of chromosomes can cause Down syndrome

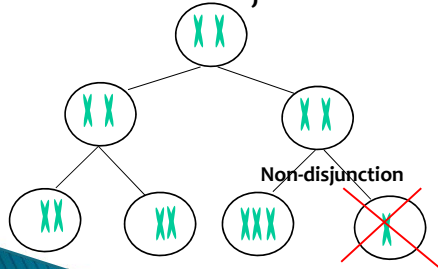


- 95% people have three separate copies of chromosome 21 - **trisomy 21**



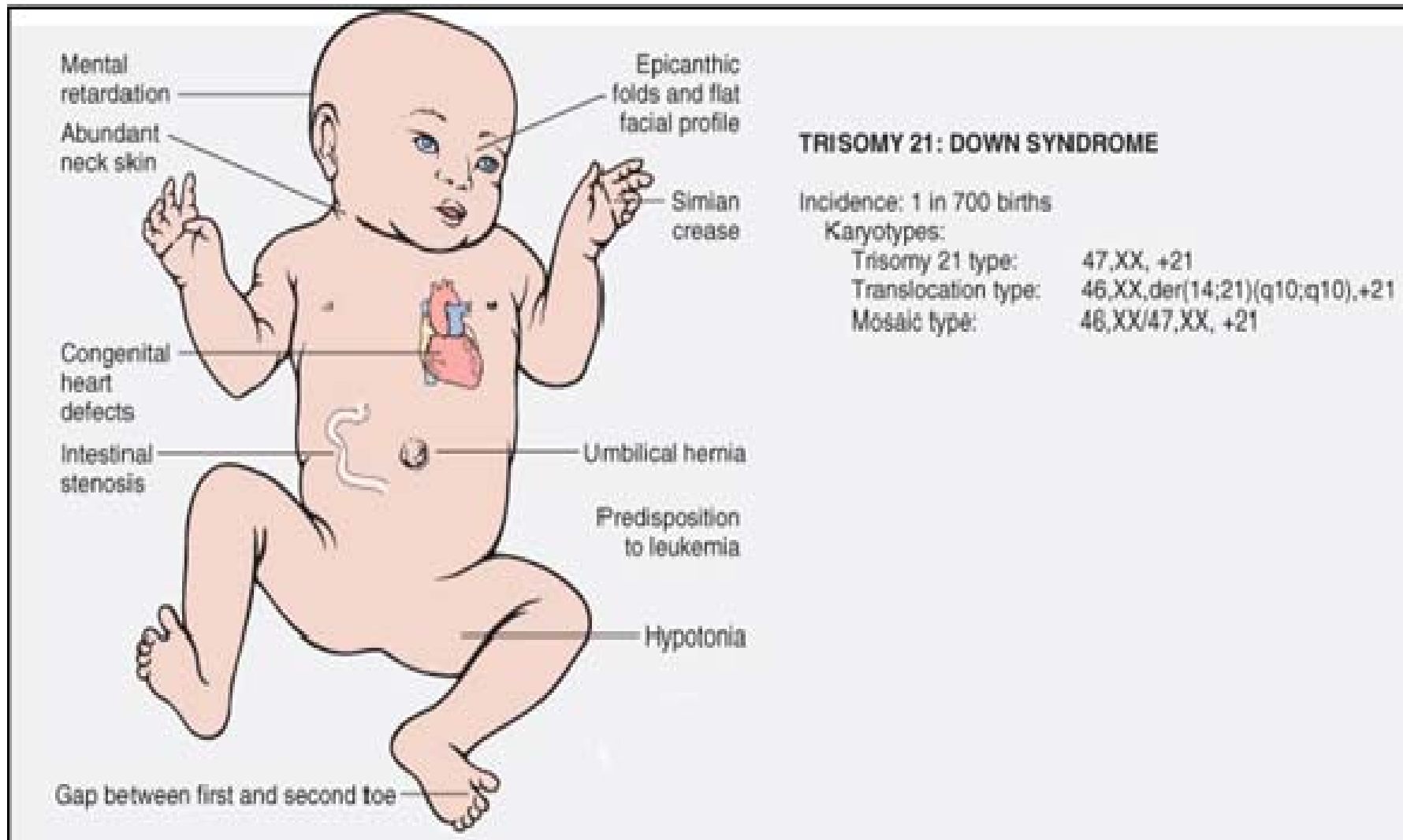
- 4% have the extra copy of chromosome 21 because of a **Robertsonian translocation**

Non-disjunction



- ▶ 1% have **mosaicism** with normal and trisomy 21 cell lines (and usually have much milder features because of the presence of the normal cells); occurs postzygotically

Clinical Features of Down Syndrome



Prenatal Diagnosis

- ▶ Amniocentesis
 - most common modality
 - performed at 15–17 weeks gestation
- ▶ Chorionic Villus Sampling (CVS)
 - second most common
 - performed at 10–12 weeks gestation
- ▶ Percutaneous umbilical blood sampling (PUBS)
 - performed in second and third trimesters
 - usually prompted by ultrasound abnormalities of fetus



Two other important autosomal trisomies

- ▶ **Edwards syndrome** (trisomy 18)
 - 1 in 3000 births
 - multiple malformations (especially heart, kidneys)
 - clenched hands with overlapping fingers

- ▶ **Patau syndrome** (trisomy 13)
 - 1 in 5000 births
 - multiple malformations
 - affects midline structures particularly:
incomplete lobation of brain; cleft lip; congenital heart disease

Both syndromes have a very poor prognosis: majority of babies dying in first few weeks of life. If a baby survives (very unusual) there is severe mental retardation.



Patau syndrome



Description

- Patau syndrome – also known as trisomy 13 and trisomy D.
- Affects about 1 in 12,000 live births.
- More than 80% of infants with Patau syndrome die within their first year of life.



The Simian line, or an abnormal palm pattern that is usually a symptom of Patau syndrome.

History

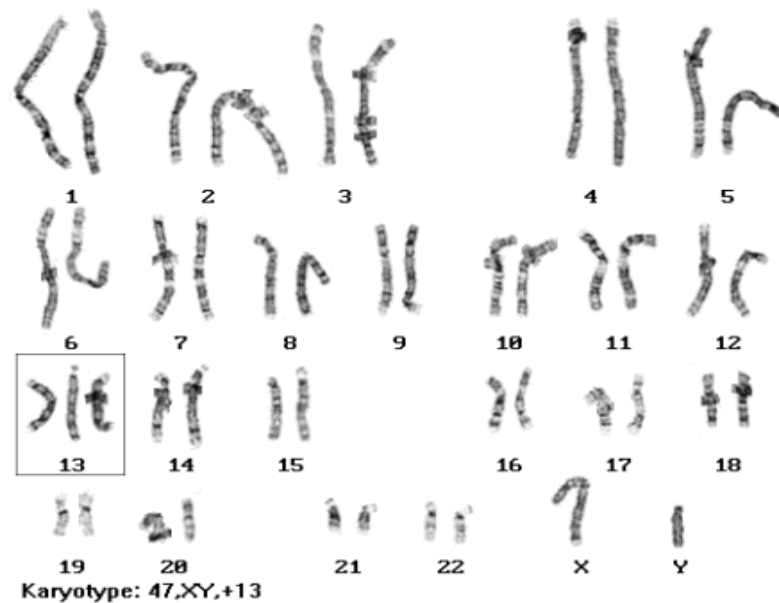
First observed by Thomas Bartholin in 1657.

However, the actual genetic and chromosomal aspects were discovered by Dr. Klaus Patau in 1960, hence the name “Patau syndrome”.

Cause

The cells have three copies of chromosome 13 instead of the normal two, as well as extra material from the extra chromosome attached to another chromosome, resulting in changes.

Most cases occur as random events during the formation of gametes – An error in meiosis.



Mosaic Patau

A small percentage of cases occur when only some of the body's cells have an extra copy of chromosome 13, resulting in a mixed population of cells with differing numbers of chromosomes. This is called Mosaic Patau.



A baby with a cleft palate, a common abnormality of Patau syndrome.

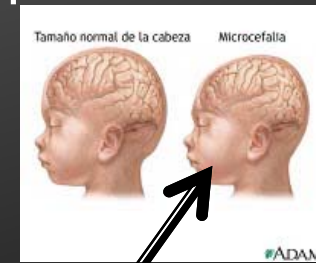
Common Problems

Nervous system problems:

- Mental and motor disabilities similar to that of autism
- Microcephaly, or a less rounded brain resulting in more of an egg-shaped skull
- Eye structure defects:
 - Microphthalmia, or crossed eyes (may involve one eye or both)
 - Cataracts
 - Sensory Nystagmus, or involuntart “twitching” of the eye
 - Optic nerve hypoplasia, or the underdevelopment of the optic nerve



Sensory nystagmus



Common Problems cont.

Muscular and skin problems:

- Polydactyly, or extra fingers/toes
- Low-down ears
- Prominent heels and deformed feet, called 'rocker-bottom' feet
- Strange palm patterns, commonly called the Simian line
- Overlapping of the fingers over thumb
- Cleft palate



Polyd



The Simian line



'Rocker-bottom' feet

Common Problems, cont.

Vascular Problems:

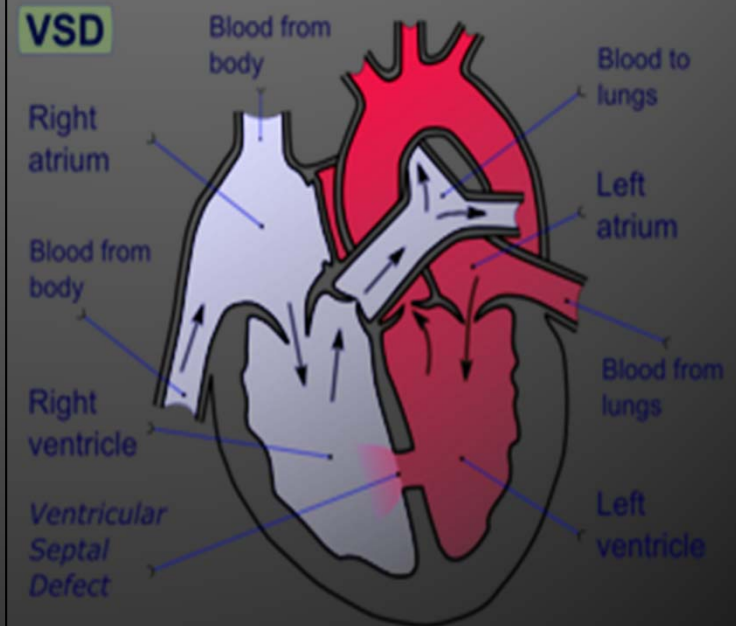
- Kidney problems
- Heart defects such as ventricular septal defect



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Kidney Problem

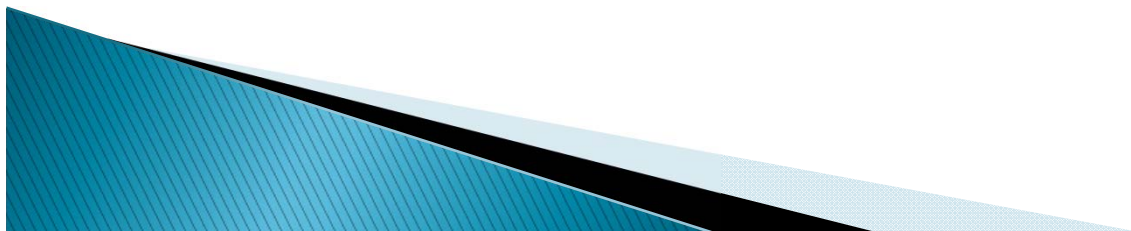
The disease shown right is called Polycystic kidney disease (PKD). This is a disorder in which clumps of cysts develop within your kidneys. Cysts are small round sacs containing water-like fluid.



Treatment

- Since most infants with Patau syndrome die within the first year of life, special management/procedures are necessary to fix defects to allow the child to survive for as long as possible

Chromosome 22q11 deletion syndrome



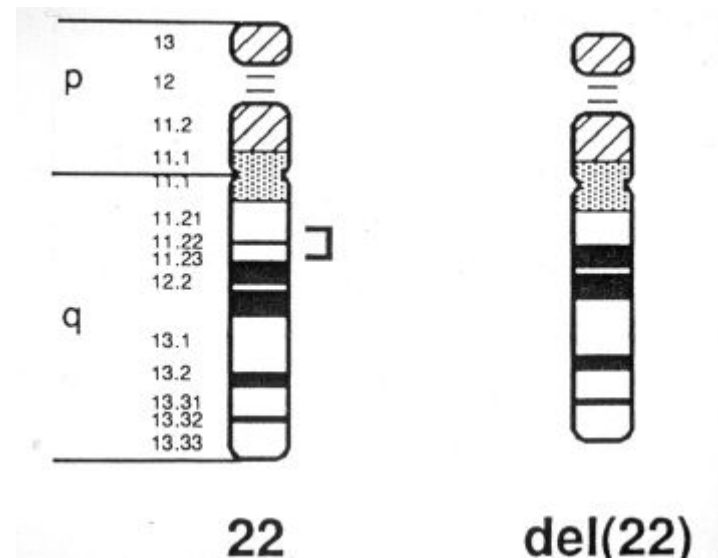
Chromosome 22q11 deletion syndrome

- ▶ DiGeorge Syndrome,
- ▶ Velo(soft palate)Cardio(heart)Facial(face) Synd
- ▶ Conotruncal anomaly face syndrome
- ▶ Congenital Thymic Aplasia,
- ▶ mnemonic C-A-T-C-H:
 - Cardiac Abnormality (especially Fallot's Tetralogy)
 - Abnormal facies
 - Thymic aplasia
 - Cleft palate
 - Hypocalcemia



Chromosome 22q11.2 Deletion Syndrome

- ▶ Because of a DELETION, this cannot be detected by standard karyotyping and needs FISH



- ▶ Cytogenetic abnormalities involving sex chromosome

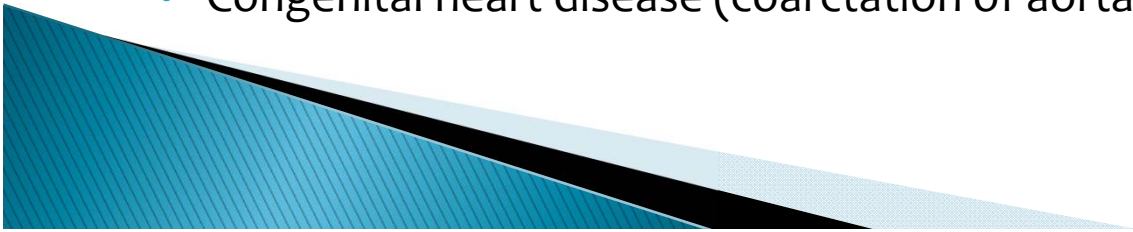


Two important conditions caused by anomalies of sex chromosome number

▶ Klinefelter syndrome

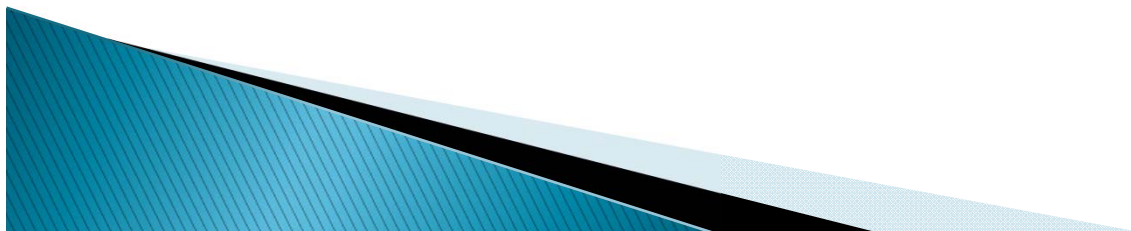
- 47,XXY
- 1 in 1000 males
- Infertility (atrophic testes do not produce sperm)
- Poorly developed 2ndy sexual characteristics in some (lack of testosterone)
- Tall

▶ Turner syndrome

- 45,X
 - 1 in 5000 females
 - 99% are lost spontaneously in pregnancy
 - Short stature
 - Primary amenorrhoea (ovaries involute before birth)
 - Congenital heart disease (coarctation of aorta) 20%
- 

SEX CHROMOSOME DISORDERS

- ▶ Problems related to sexual development and fertility
- ▶ Discovered at time of puberty
- ▶ Retardation related to the number of X chromosomes
- ▶ If you have at least ONE “Y” chromosome, you are male



X-Chromosomal Disorders

- ▶ Imbalances of X-chromosomes are better tolerated than those of autosomes
- ▶ Lyonization – Mary Lyon
 - during 16th day of embryonic life one X-chromosome in females is randomly inactivated
 - inactivation persists in all subsequent cells
- ▶ Increased number of X-chromosomes in either males or females lead to mental retardation



KLINEFELTER (XXY, XXXY, etc.)

- ▶ Hypogonadism found at puberty
- ▶ **#1 cause of male infertility**
- ▶ NO retardation unless more X's
- ▶ 47, XXY 82% of the time
- ▶ L----O----N----G legs, atrophic testes. small penis



Klinefelter Syndrome

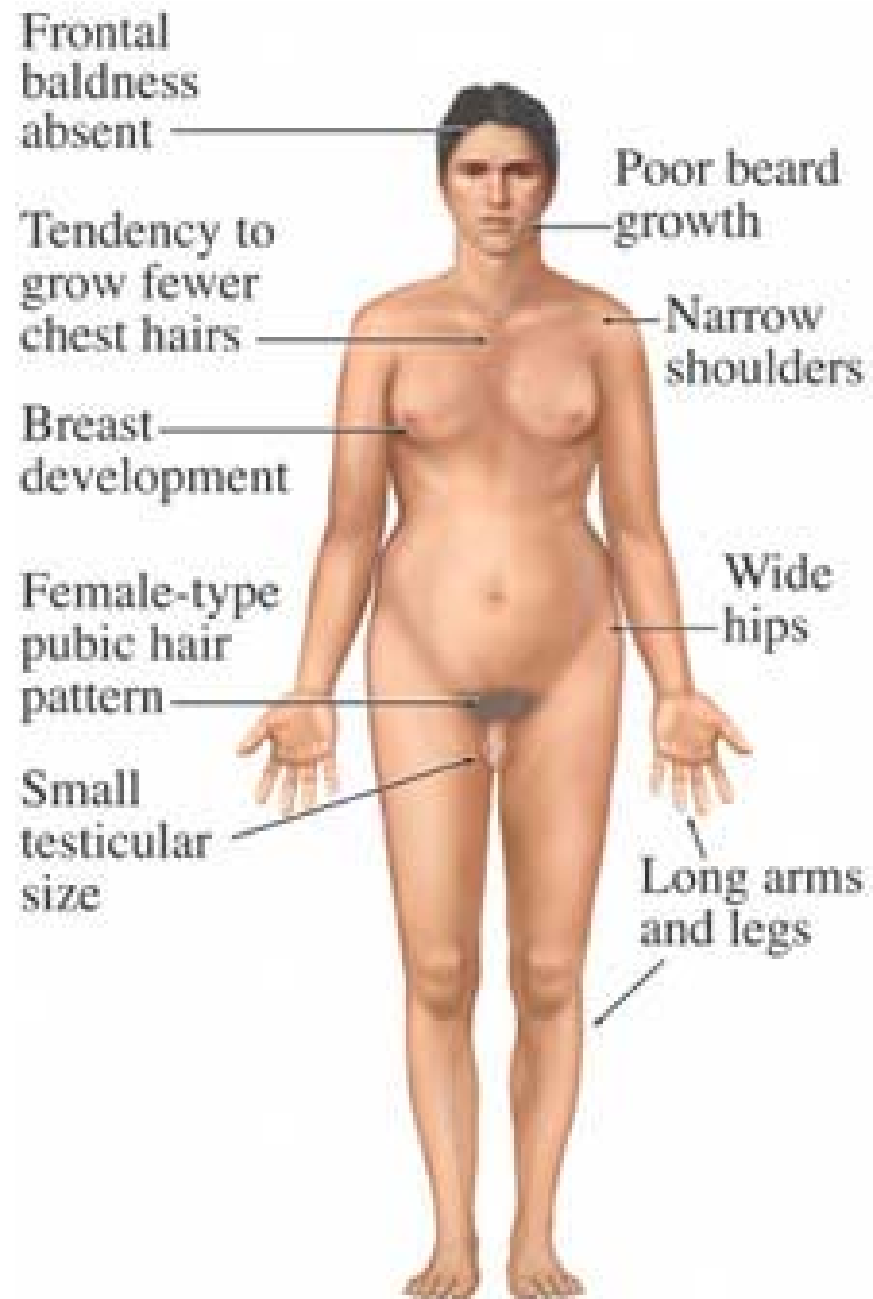
- ▶ *A male hypogonadism that occurs when there are two or more X-chromosomes and one or more Y-chromosomes*
- ▶ Incidence is 1 in 500 male births
- ▶ Usually (82% of cases) 47,XXY
 - maternal (60%) or paternal (40%) nondisjunction during meiotic divisions
- ▶ 15% are mosaics, usually 46,XY/47,XXY



Clinical Features

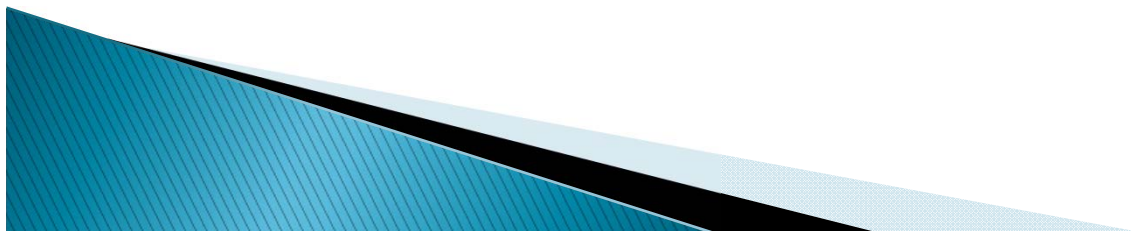
- ▶ Testicular abnormality does not develop before puberty
 - seminiferous tubules are atrophic resulting in reduced spermatogenesis, infertility, small firm testes, and increased FSH
 - testosterone levels are reduced
 - impotence and increased LH
 - lack of secondary male sexual characteristics
- ▶ Mental retardation is unusual but IQ may be below normal
- ▶ Mosaics are less severely affected





TURNER (XO)

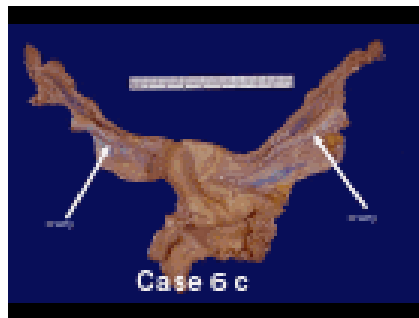
- ▶ 45, X is the “proper” designation
- ▶ Mosaics common
- ▶ Often, the WHOLE chromosome is not missing, but just part
- ▶ NECK “WEBBING”
- ▶ EDEMA of HAND DORSUM
- ▶ CONGENITAL HEART DEFECTS most FEARED



Turner Syndrome

- ▶ Results from complete or partial monosomy of the X-chromosome in females
- ▶ Most common sex chromosome abnormality in females, incidence 1 in 1000 live births
- ▶ Classical cytogenetics
 - 45,X (57%)
 - structural abnormalities of X-chromosomes (14%)
 - mosaics (29%)





Short stature

Low hairline

Shield-shaped thorax

Widely spaced nipples

Shortened metacarpal IV

Small finger nails

Brown spots (nevi)

Characteristic facial features

Fold of skin

Constriction of aorta

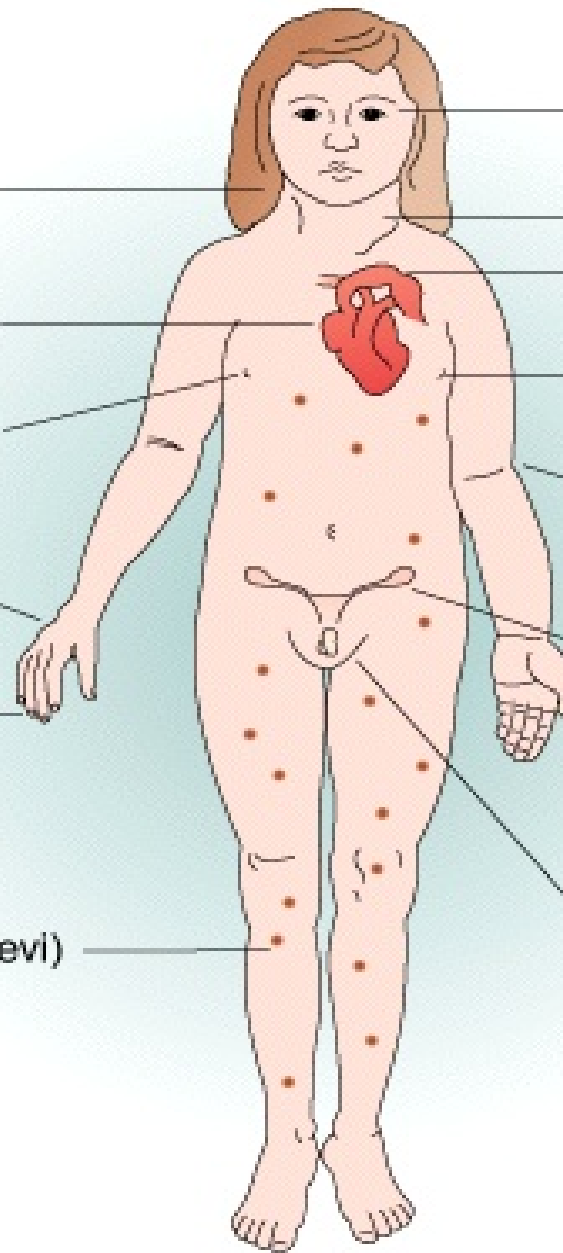
Poor breast development

Elbow deformity

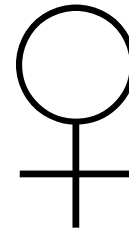
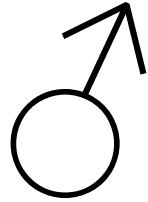
Rudimentary ovaries

Gonadal streak (underdeveloped gonadal structures)

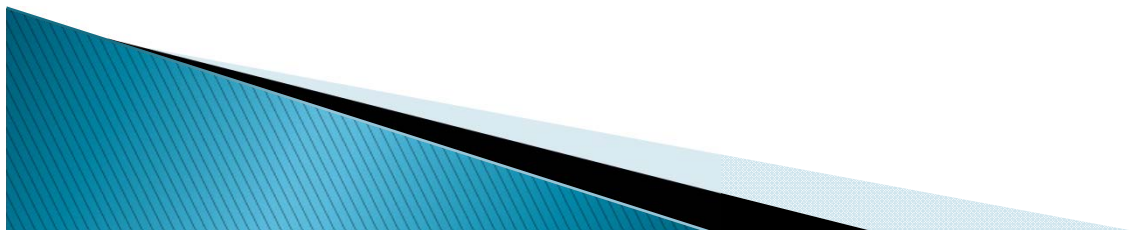
No menstruation



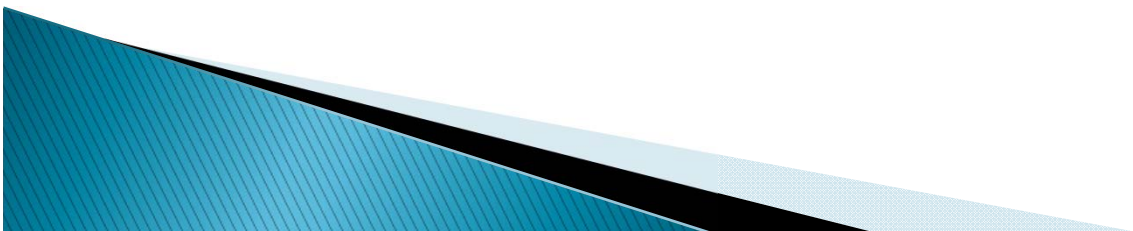
HERMAPHRODITES



- ▶ **GENETIC SEX** is determined by the **PRESENCE** or **ABSENCE** of a “Y” chromosome
- ▶ **TRUE HERMAPHRODITE: OVARIES AND TESTES, often on opposite sides (VERY RARE)**
- ▶ **PSEUDO-HERMAPHRODITE:**
 - **MALE: TESTES** with female characteristics (Y-)
 - **FEMALE: OVARIES** with male characteristics (XX)

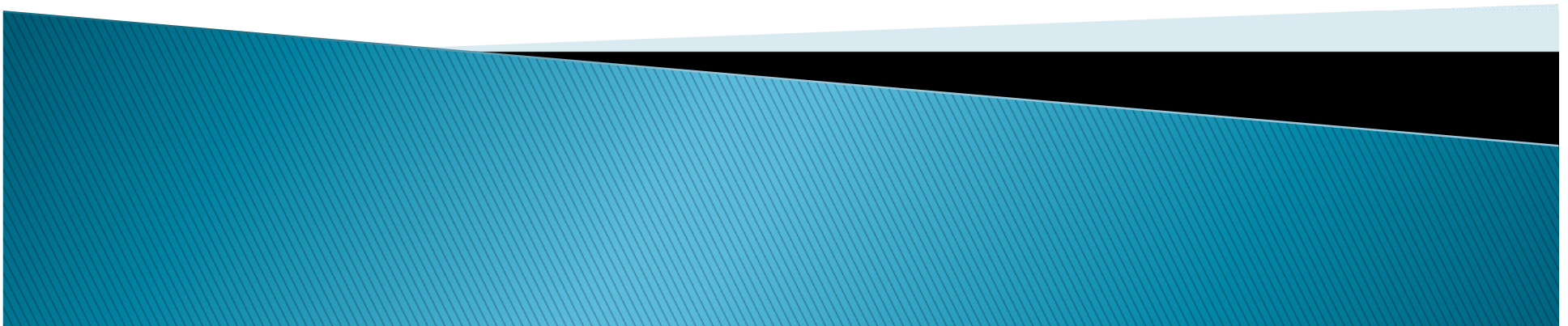


► Thank you



Other genetic disorders and diagnosis

Class 4



SINGLE GENE, NON-Mendelian

- ▶ **Triplet repeats**
 - Fragile X (CGG)
 - Others: ataxias, myotonic dystrophy
- ▶ **Mitochondrial Mutations: (maternal)**
(LEBER HEREDITARY OPTIC NEUROPATHY)
- ▶ **Genomic “IMPRINTING”:** (Inactivation of maternal or paternal allele, contradicts Mendel)
- ▶ **Gonadal “MOSAICISM”:** (only gametes have mutated cells)



Triplet Repeat Disorders

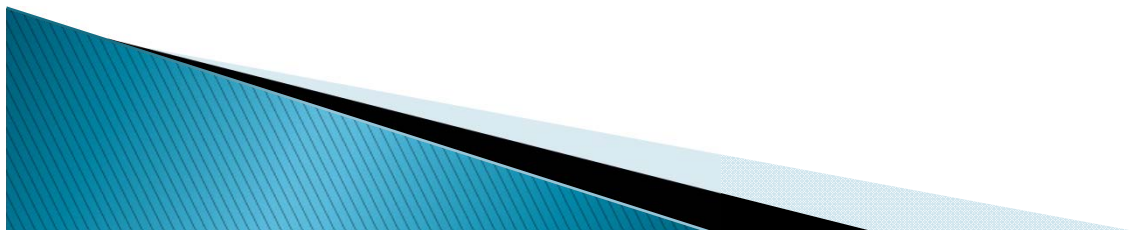
Fragile-X syndrome

- ▶ Triplet expansion consists of repeating sequences of 3 nucleotides, usually including guanine (G) and cytosine (C) and may occur in coding or noncoding regions of the gene
- ▶ Triplets undergo expansion during gametogenesis
- ▶ Above a certain threshold of repeats, function is impaired and disease results
- ▶ Currently comprise 20 diseases



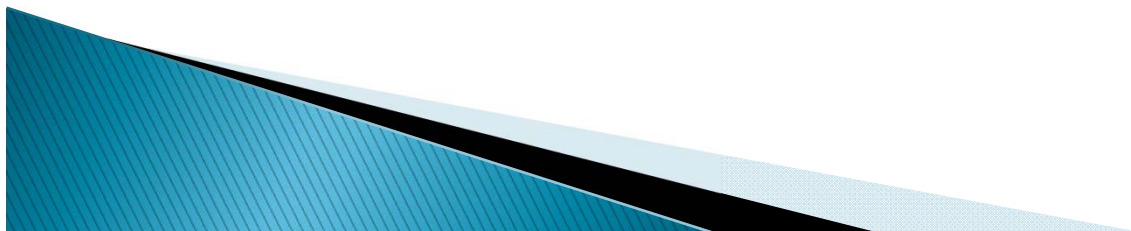
Fragile X syndrome

- ▶ Affects males
- ▶ Long face
- ▶ Large mandible
- ▶ Large everted ears
- ▶ Large testicles (90%)
- ▶ Some pts–
 - ▶ Hyperextensible joints, high arched palate, mitral valve prolapse



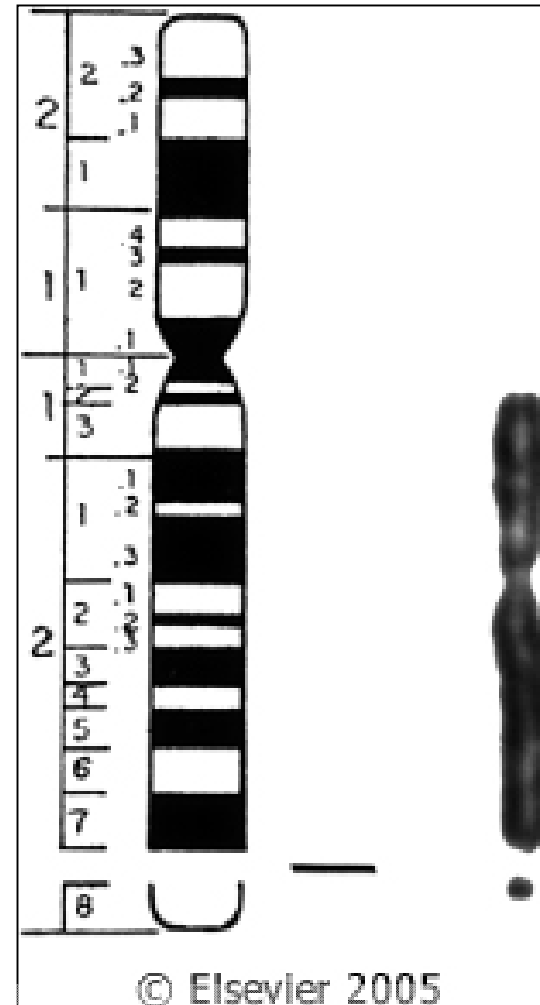
Fragile X Syndrome

- ▶ Second most common cause of mental retardation (after Down's syndrome)
- ▶ Mutation is present in an untranslated portion of the Familial Mental Retardation Gene (FMR-1)
- ▶ Loss of function mutation



Fragile-X

Cytogenetic abnormality appears as a constriction in the long arm of X-chromosome

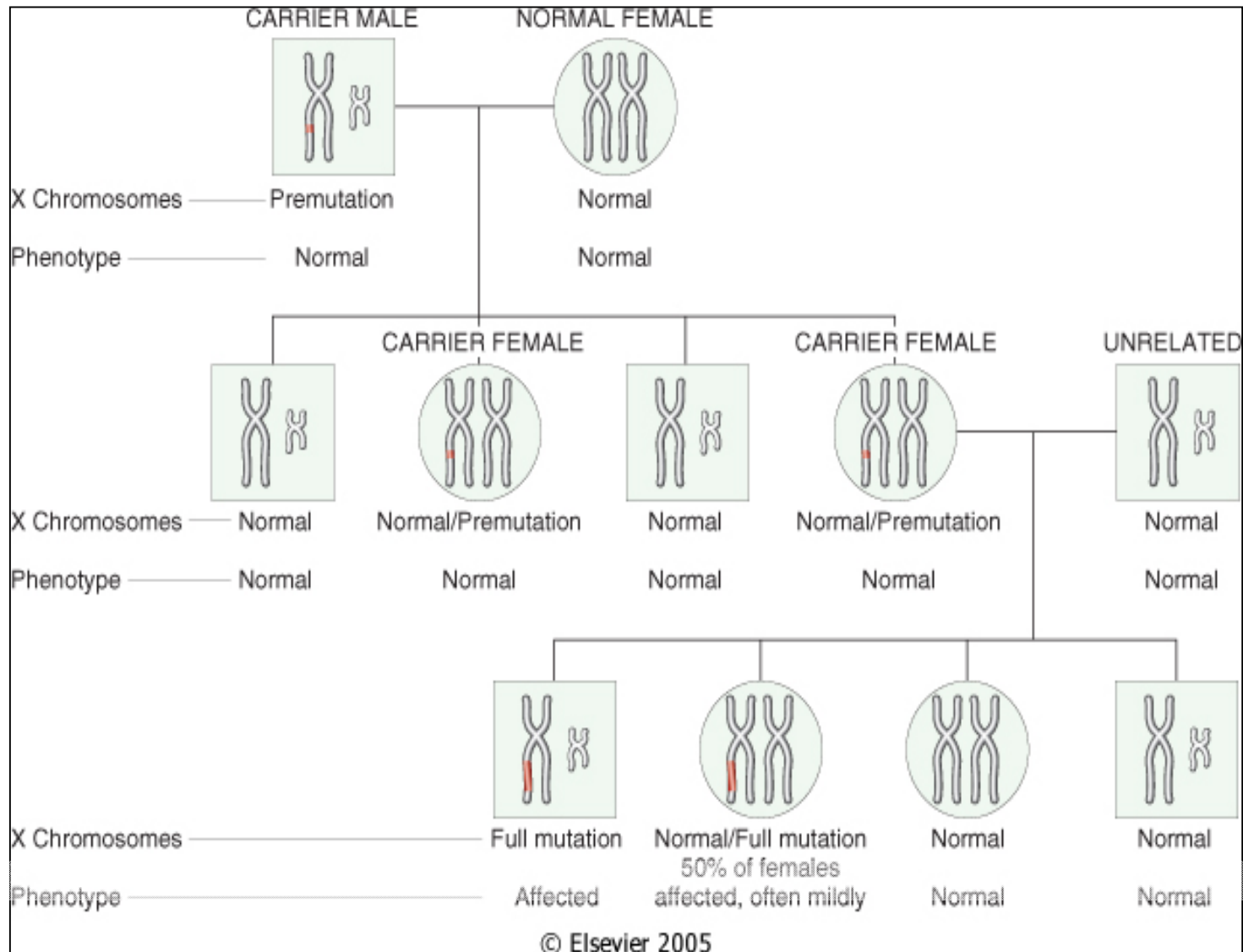


Fragile X Transmission

- ▶ Male carrier
 - detected by pedigree analysis and genetic tests
 - 20% are clinically and cytogenetically normal
- ▶ Daughters are obligate carriers
 - 50% are affected (i.e. retarded)
 - transmit disease to grandsons of male carrier
- ▶ Risk of phenotypic effects
 - depends on position in pedigree
 - Sherman paradox
- ▶ Anticipation
 - defect worsens with each successive generation



Fragile-X Pedigree



Fragile X Diagnosis

- ▶ Cytogenetics
- ▶ PCR based molecular methods of repeats in FMR-1 gene
 - normal – 10 – 55 repeats
 - premutation – 55 – 230 repeats in carrier state
 - mutation – 230 – 4000 in full clinical syndrome
- ▶ Affected females
 - unfavorable lyonization

Sherman's paradox: likelihood of MR is much higher in grandsons than in brothers of transmitting males



Huntington Disease

- ▶ Autosomal trinucleotide repeat disorder
- ▶ Expansion occurs during spermatogenesis
- ▶ Expansion occurs in exons coding the highly conserved cytoplasmic protein huntingtin
- ▶ Toxic gain in function produces
 - movement disorder
 - dementia



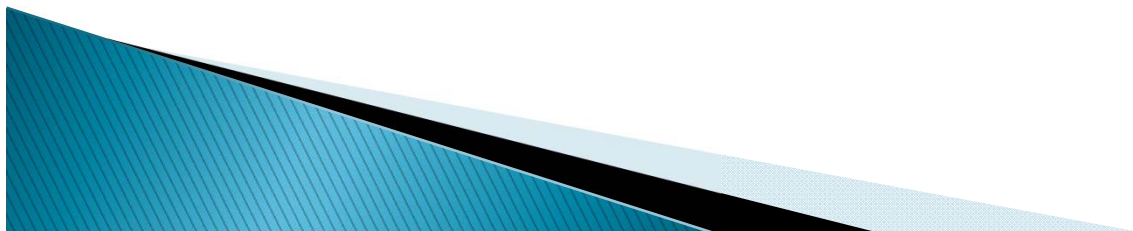
Mitochondrial mutations

- Mitochondria have their own DNA, encoding for proteins involved mainly in oxidative metabolism
- These genes are derived from the ovum, since the sperm has little cytoplasm and few mitochondria
- Inheritance is via the mother



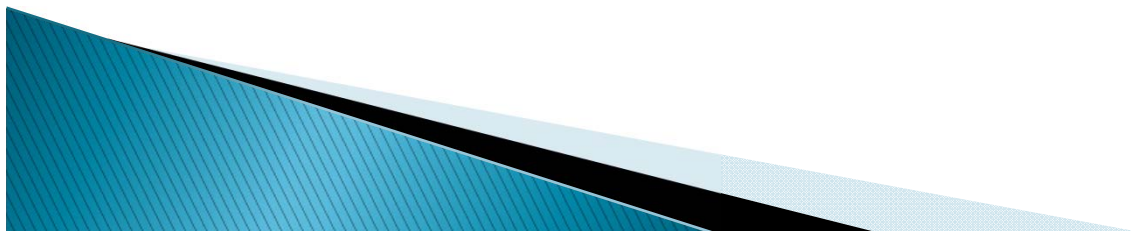
Leber's Hereditary Optic Neuropathy (LHON)

- ▶ Leber optic atrophy
- ▶ mitochondrially inherited (mother to all offspring)
degeneration of retinal ganglion cells (RGCs) and their axons
- ▶ Progressive loss of bilateral vision; blindness
- ▶ Cardiac defects
- ▶ Minor neurological manifestations
- ▶ affects predominantly young adult males.



Genomic imprinting

- ▶ is a genetic phenomenon by which certain genes are expressed in a **PARENT-OF-ORIGIN** specific manner.
- ▶ Selective inactivation of either maternal or paternal allele before fertilization
- ▶ Transmitted to all somatic cells



Genomic imprinting

Prader–Willi syndrome

- ▶ del of band q12 on chromo 15 (paternal)
- ▶ Mental retardation, short stature, hypotonia, obesity, small hands and feet, hypogonadism

Angelman syndrome

- ▶ del of same chromo region from mother
- ▶ MR, ataxic gait, seizures, inappropriate laughter – happy puppets



Molecular diagnosis

► Applications:

- Detection of inherited mutations
- Detection of acquired mutations
- Accurate diagnosis and classification of neoplasms
- Diagnosis of infectious diseases
- Determination of identity, transplantation, paternal testing, forensic medicine



Indications of prenatal chromosome analysis

- ▶ Advanced maternal age >34 yrs
- ▶ A previous child with a chromosomal abnormality
- ▶ A carrier parent of X-linked genetic disorder
- ▶ A carrier parent of translocation or inversion



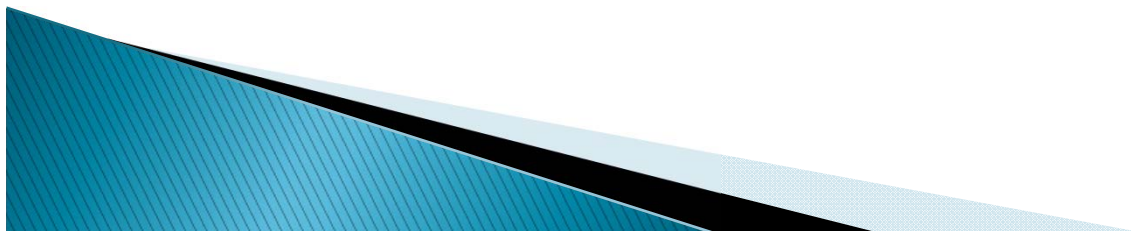
Indications of post natal chromosome analysis

- ▶ Multiple congenital abnormalities
- ▶ Unexplained mental/developmental retardation
- ▶ Suspected aneuploidy (down's)
- ▶ Suspected unbalanced autosome (imprinting)
- ▶ Suspected sex chromosome abnormality
- ▶ Suspected fragile X syndrome
- ▶ Infertility (to rule out sex chromo abn)
- ▶ Multiple spontaneous abortions (both partners)



Diagnosis of genetic diseases

- ▶ Cytogenetic analysis: Karyotyping
- ▶ Molecular analysis



Karyotyping

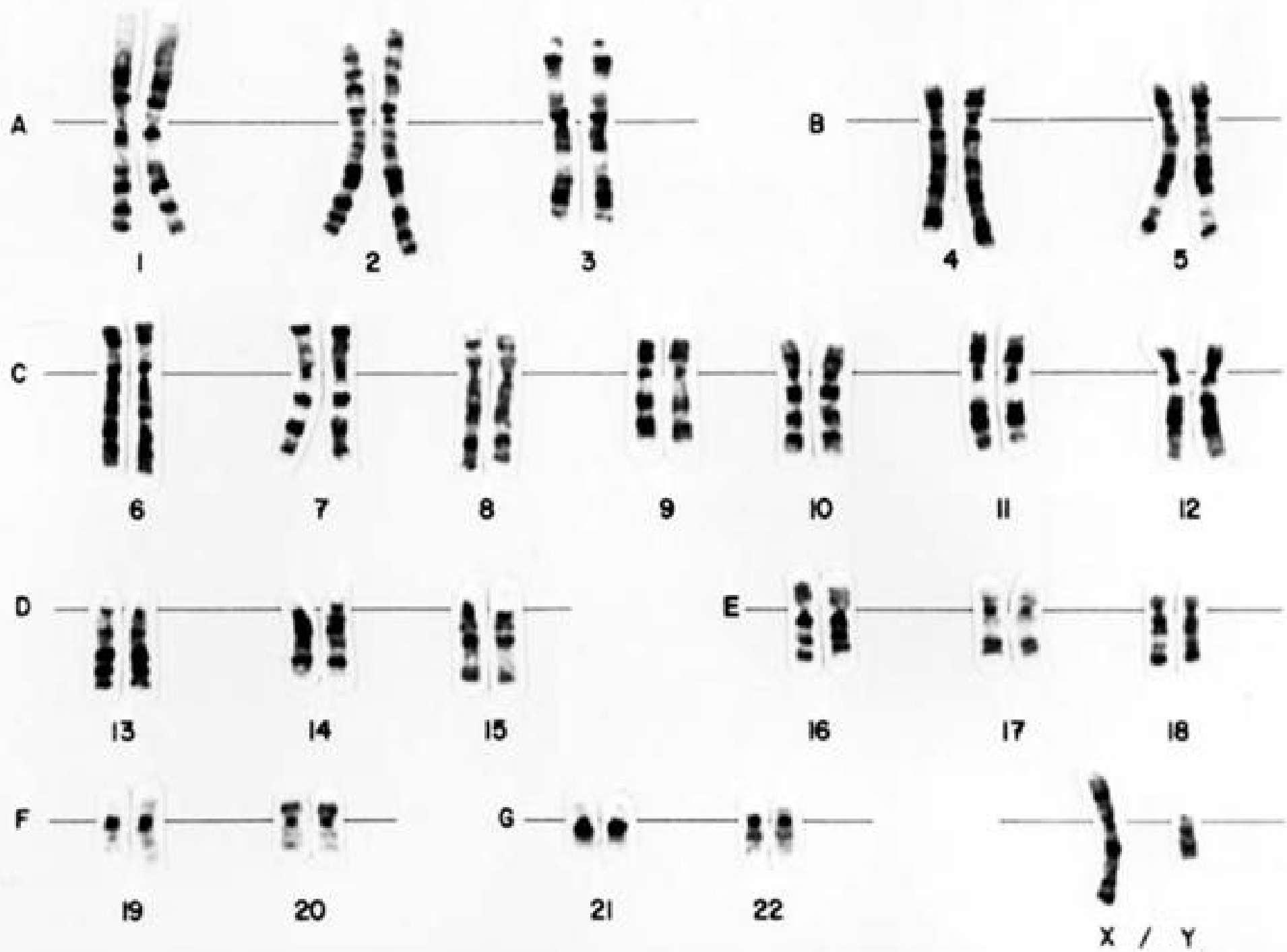
- ▶ Arrest of mitosis in metaphase by colchicine followed by staining of chromosomes
- ▶ In interphase cells, the genetic material is dispersed and chromosomes are not visible
- ▶ Identification of each chromo on pattern of distinctive light and dark bands
- ▶ Giemsa stain – G banding

Described as: 47,XY, +21 (Downs)

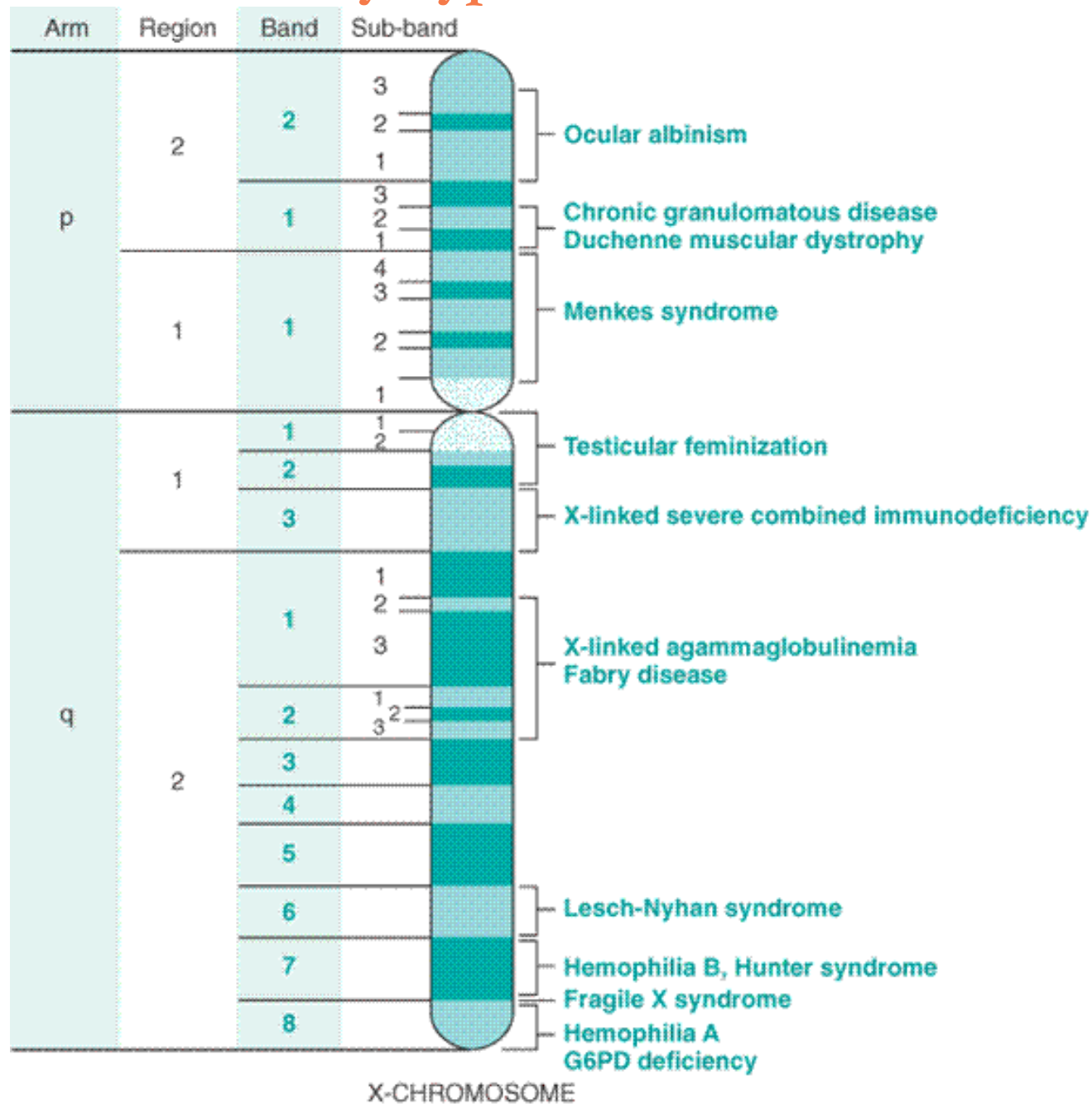
- ▶ Total no of chromo, sex chromo, abnormality
- ▶ Short arm – p
- ▶ Long arm – q



- A,B,C,D,E,F,G depends on chromosome length
 - A longest
 - G shortest
- **ARM→REGION→BAND→Sub-BAND**,
numbering from the centromere progressing
distally



Banded karyotype of X chromosome



Routine banded karyotype detects

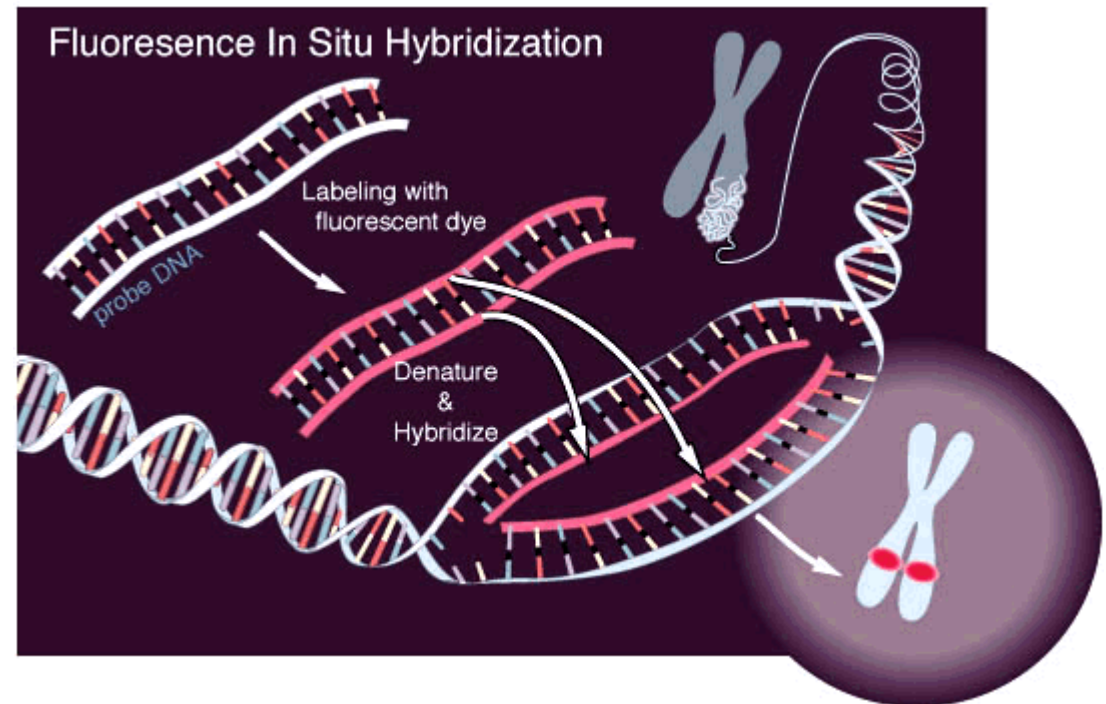
- **Abnormal number of chromosomes**
- **Large duplications and deletions**
- **Balanced rearrangements (translocations, inversions)**

F.I.S.H. (gene “probes”) greatly enhances G-banding

- **F**luorescent **I**n-
Situ

- H**ybridization

- Uses fluorescent labelled DNA fragments, ~10,000 base pairs, to bind (or not bind) to its complement



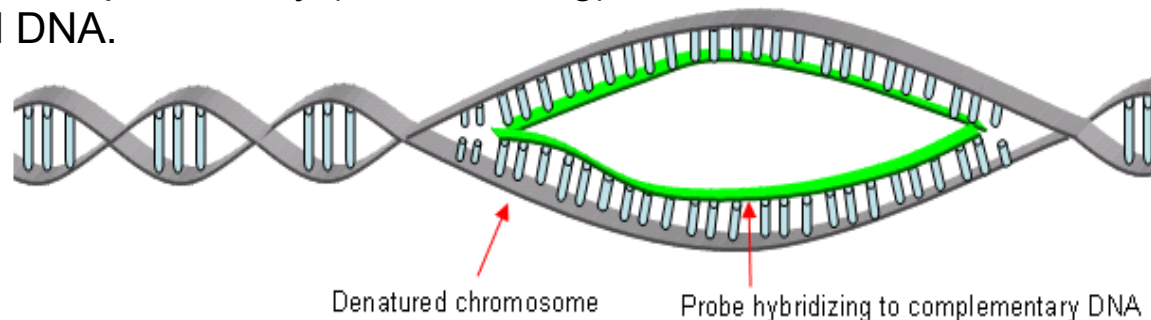
FISH

fluorescent in situ hybridization: (FISH) A technique used to identify the presence of specific chromosomes or chromosomal regions through hybridization (attachment) of fluorescently-labeled DNA probes to denatured chromosomal DNA.

Step 1. Preparation of probe. A probe is a fluorescently-labeled segment of DNA complementary to a chromosomal region of interest.



Step 2. Hybridization. Denatured chromosomes fixed on a microscope slide are exposed to the fluorescently-labeled probe. Hybridization (attachment) occurs between the probe and complementary (i.e., matching) chromosomal DNA.

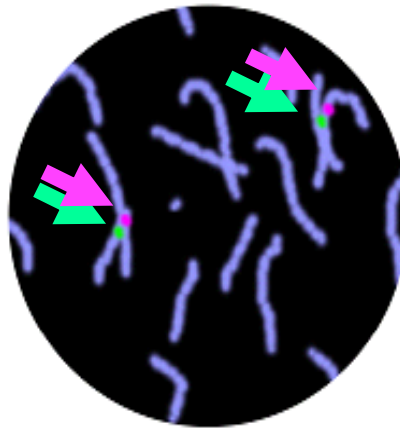


FISH

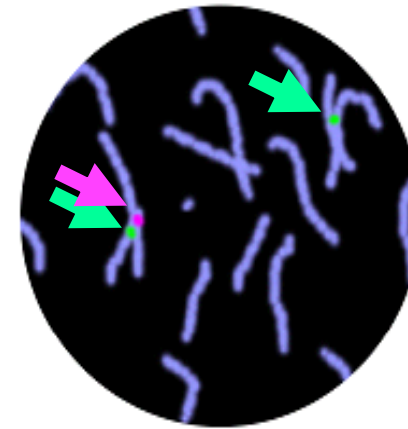
Step 3. Visualization. Following hybridization, the slide is examined under a microscope using fluorescent lighting. Fluorescent signals indicate the presence of complementary chromosomal DNA; absence of fluorescent signals indicate absence of complementary chromosomal DNA.

Green signal =
Normal control

Pink signal =
Chromosome region of interest



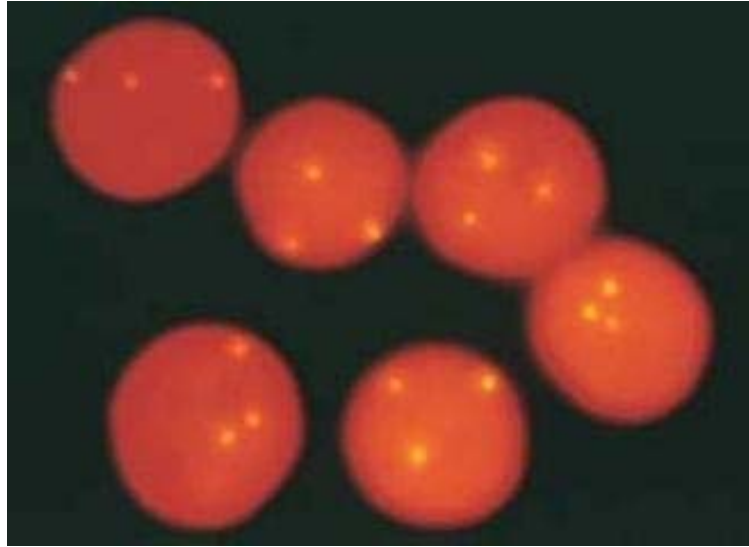
Normal control:
Two green signals
Two pink signals



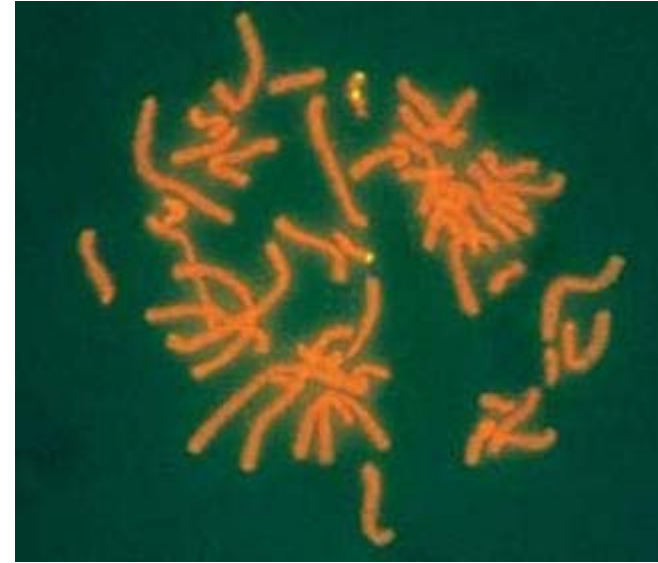
Patient with deletion:
Two green signals
One pink signal

FISH detects small (submicroscopic) chromosome

- **Deletions**
- **Duplications**
- **Translocations**
- **Is applicable to interphase cells**

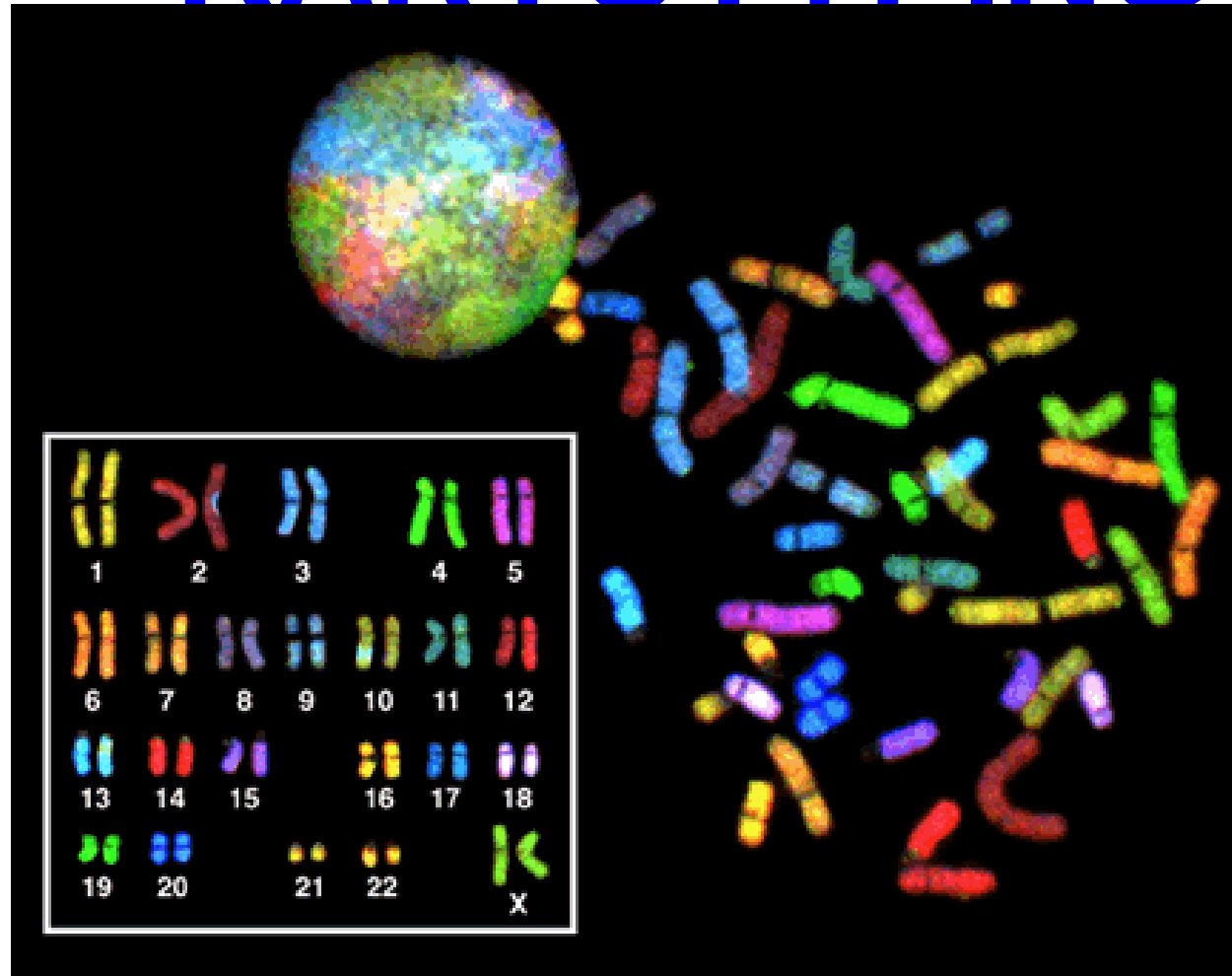


**TRIPLE CHROMOSOME
#20**



**A DELETION in
CHROMOSOME #22**

SPECTRAL KARYOTYPING

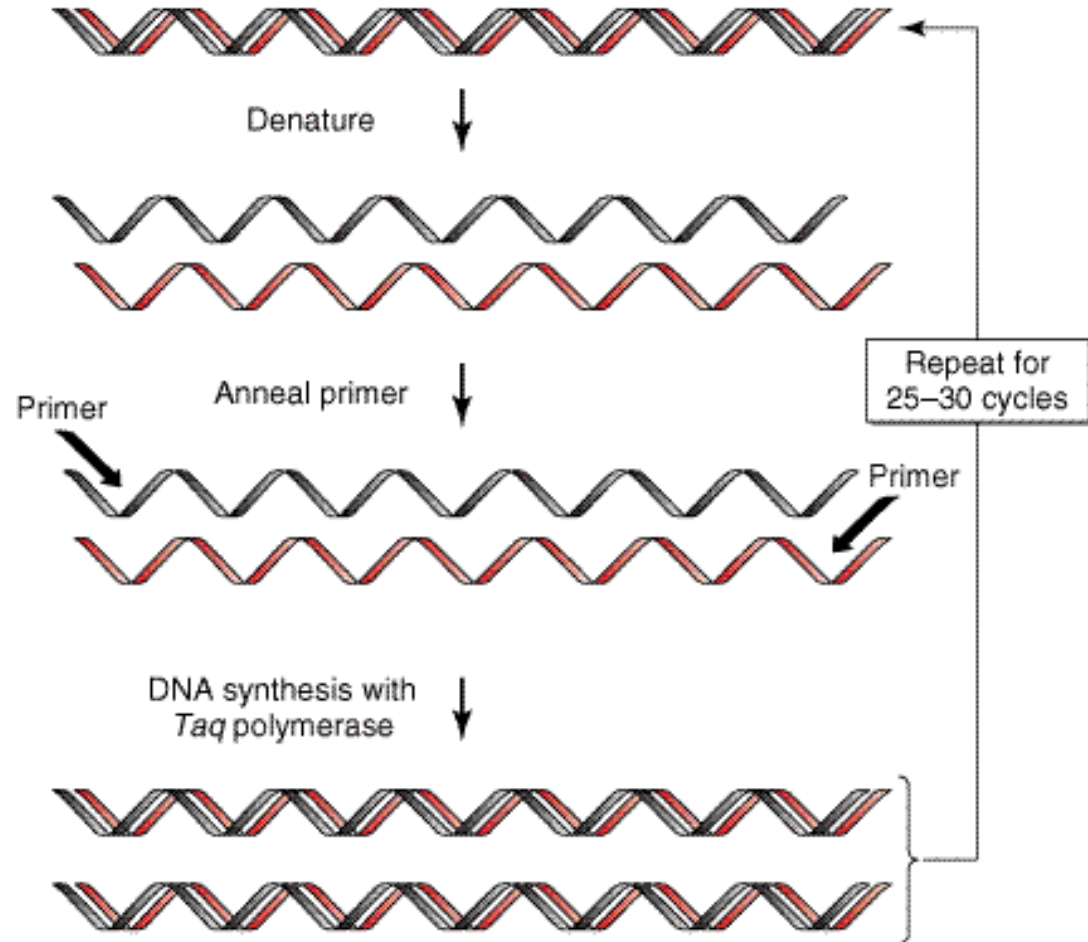


Diagnosis of single gene disorders by recombinant DNA

- Direct gene diagnosis
- Indirect DNA diagnosis: linkage analysis

Polymerase Chain Reaction

Figure 56-7



Direct gene diagnosis

1. Some mutations alter certain restriction sites on DNA .eg mutations in factor V
 - 2 primers which bind to 3' and 5' ends of a normal allele are produced.
 - DNA between the primers is amplified
 - Then digested by MnlI enzyme
 - Mutant gene- 2 products
 - Normal gene – 3 products

Direct gene diagnosis

2. Allele specific oligonucleotide hybridization

- Mutation does not alter cutting site of enz
- Control and pt DNA amplified using primers that flank mutation site
- Each sample applied on a filter paper as a dot
- 2 oligonucleotides which have at their centre single base by which normal and mutant genes differ are produced and radiolabelled
- Allowed to hybridize with control & pt DNA
- Normal pts- strong signal
- Hetrozygotes – faint signal
- Homozygotes – no signal

Direct gene diagnosis

- Mutations affecting length of DNA
 - 2 primers that flank the region affected by trinucleotide repeats (in fragile X) amplify the intervening sequences
 - Differential migration of amplified DNA products on a gel according to size

Indirect DNA diagnosis: linkage analysis

- When information about gene sequencing is lacking in a disease
- To differentiate abnormal chromosome from normal based on polymorphisms (variations) in DNA sequences
 - Site polymorphism
 - Length polymorphism

Indirect DNA diagnosis: linkage analysis

Site polymorphism

- Restriction fragment length polymorphisms
 - Single base pair changes can produce or abolish sites for restriction enzymes
 - Length of DNA fragments produced after digestion with certain enzymes is altered
 - DNA fragments of different length detected by southern blot

Indirect DNA diagnosis: linkage analysis

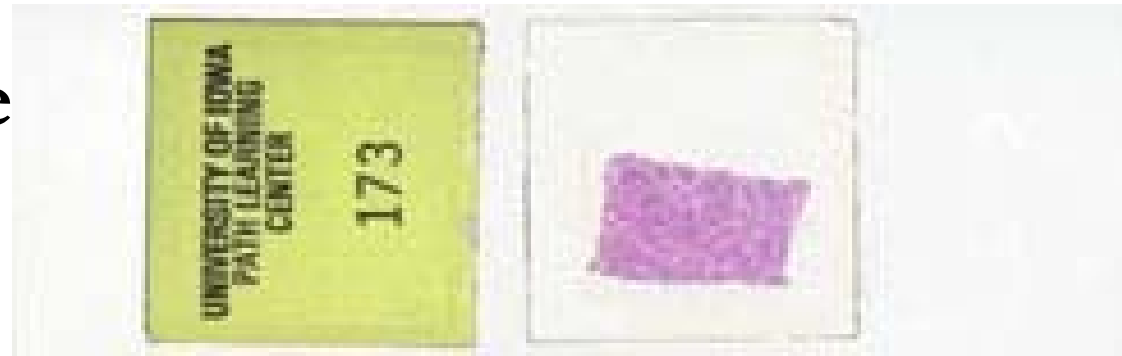
Length polymorphism

- Human DNA has short repetitive sequences of non coding DNA
- The number of repeats varies in individuals
- Microsatellite repeats- less than 1kb, 2-6bp
- Mini satellite repeats- 1-3kb, 15-70bp

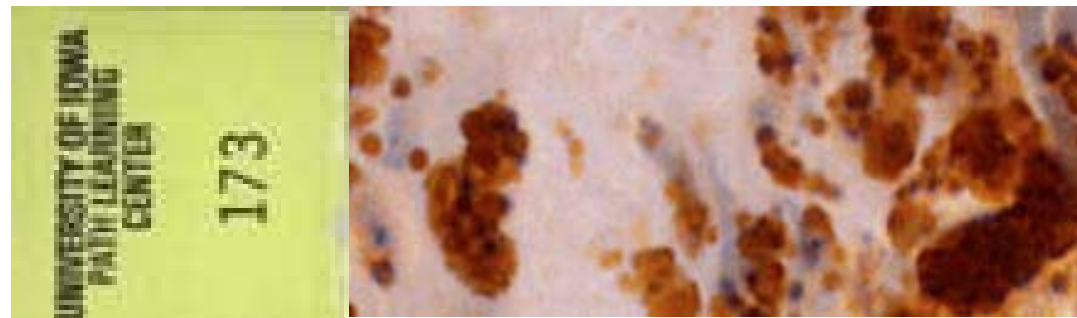
MOLECULAR DX by DNA PROBES

- **BIRTH DEFECTS, PRE- or POST- NATAL**
- **TUMOR CELLS**
- **CLASSIFICATIONS of TUMORS**
- **IDENTIFICATION of PATHOGENS**
- **DONOR COMPATIBILITY**
- **PATERNITY**
- **FORENSIC**

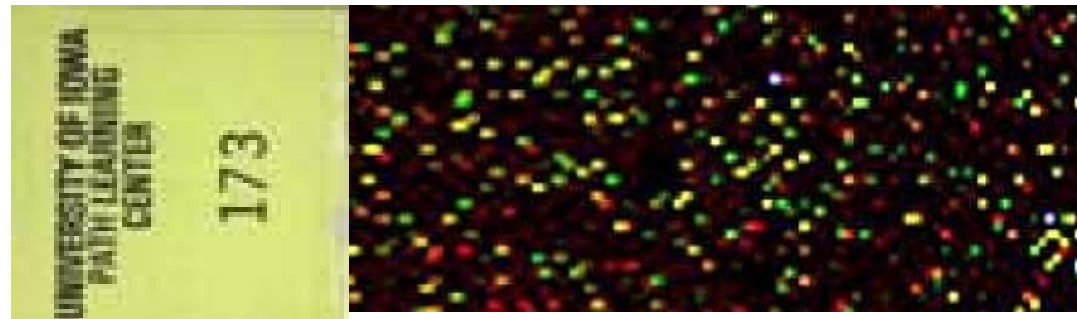
**H&E tissue
structures**



**Immuno-
Antigen
Proteins**



**GENES that
MAKE those
PROTEINS**



Thank you